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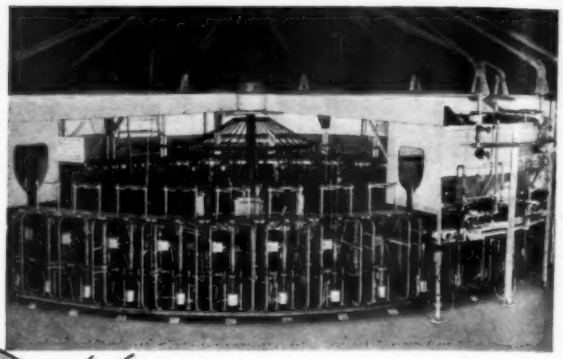

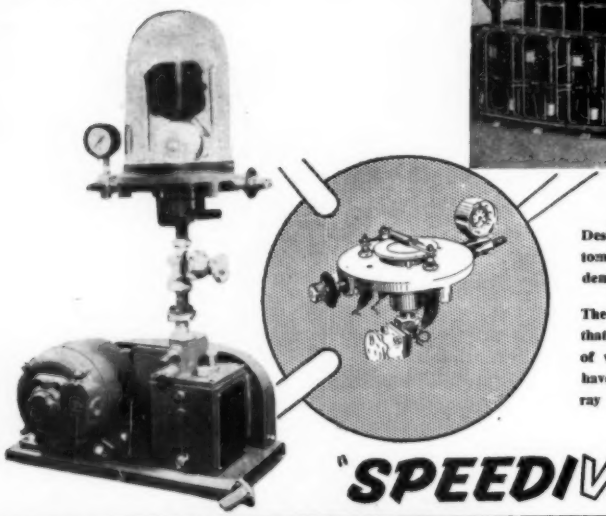

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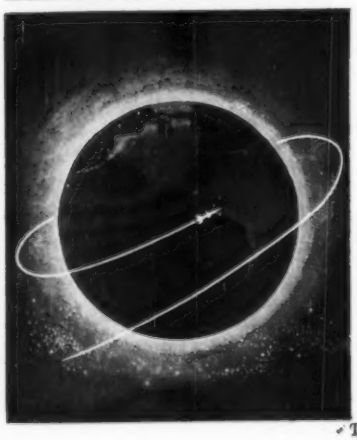

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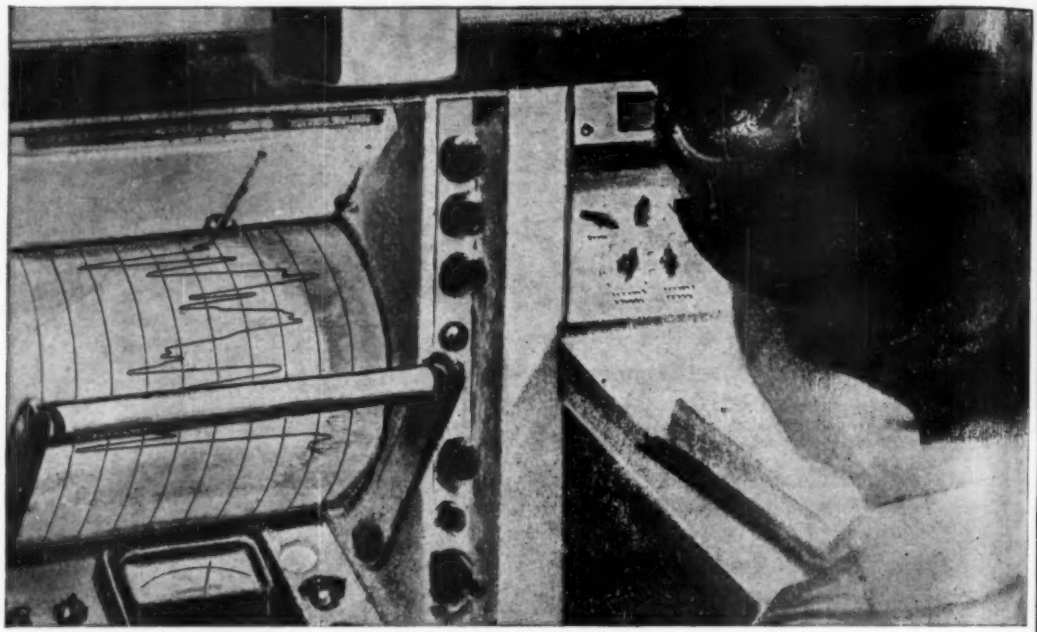

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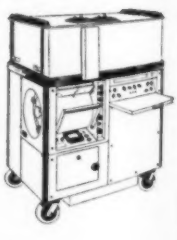

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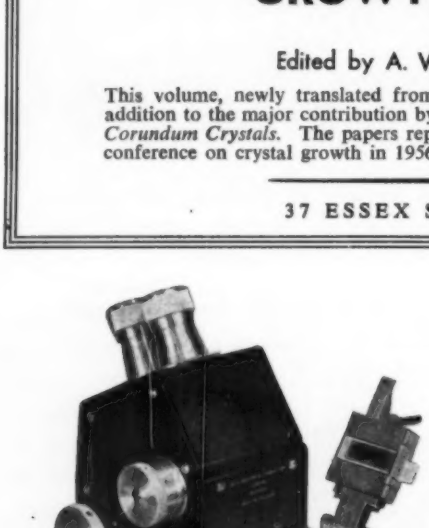

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STUDENT GRANTS FOR UNIVERSITY EDUCATION

THE report of the committee appointed in June 1958 under the chairmanship of Sir Colin Anderson "to consider the present system of awards from public funds to students attending first degree courses at universities and comparable courses at other institutions and to make recommendations" was published on June 2*. Eleven out of its sixteen members reported in favour of abolishing any means test for parents; but its key proposal is that all those ordinarily resident in Great Britain and admitted for the first time to first-degree courses in receipt of grants from the University Grants Committee should receive awards from public funds, provided, for universities in England and Wales, they had two passes at Advanced level in General Certificate of Education examinations or the equivalent; for Scottish universities they should have the statutory minimum entry qualification, that is, the 'attestation of fitness' of the Scottish Universities Entrance Board.

On July 28, the Minister of Education, Sir David Eccles, announced that, after consultation with the universities, local authorities and others concerned, the Secretary of State for Scotland and he accepted this recommendation. It was also their intention that the local authorities should continue to participate in the administration, but in view of the wide divergencies of view expressed by the public in regard to some of the Committee's recommendations, the Government needed more time to consider them. The Committee had specifically recommended that Parliament should make the Minister of Education and the Secretary of State for Scotland responsible for settling the detailed requirements, after rejecting proposals to simplify the present system either by making the universities, colleges and other institutions of higher education responsible for administering grants to students as agents of the Government, or by entrusting the award of all grants to an *ad hoc* national grants board, financed by public funds and enjoying some autonomy. The Anderson Committee recommended indeed that a Standing Advisory Committee should be appointed to advise the Ministers on decisions of policy and on problems arising in the day-to-day administration of the awards system; but while the Committee discusses in this report in some detail the advantages and drawbacks of local authorities as award-making bodies, it gives no clear lead as to the course to be followed. If the Ministry of Education and the Scottish Education Department do not assume entirely the detailed executive functions of award-making, it is suggested that these functions should be discharged by the two Departments but with the local authorities acting as their agents for some functions, or alternatively, by the local authorities, under rules drawn up by the two Ministers, subject to the students having a right of appeal to one or other of the Ministers.

From Sir David Eccles's statement it would appear that the Government intends to adopt something on the lines of the second of these three courses, possibly because it offers the best prospect of securing agreement in a field where opinions differ widely and are frequently strongly held. The Committee itself comments on the contrast between the community of interest and purpose among its witnesses and the wide diversity of their answers to the Committee's key questions. It was surprised to find how wide a difference of opinion existed, even within some well-defined groups, about the steps which should be taken to improve the present system and, except in minor matters, it was unable to detect any consensus of professional or public opinion on how to solve any of the main problems.

This is reflected to some extent in the report itself, though not, happily, in an admirable second chapter, which sets forth some general principles which should form the foundation of any system of granting awards from public funds. For this chapter alone and for the key recommendation already noted, as well as for useful but subsidiary proposals for simplification, the report is an important and valuable step which may yet lead to an agreement on major points of policy still at issue. It is a useful contribution to lucid thinking about a problem that has far-reaching implications, and not its least important feature is the way in which the reasons responsible for divergence of views among its own members on the question of the parental contribution are detailed.

First, the Committee is convinced that, while Britain urgently needs the greatest possible number of highly educated men and women, it should not depart from the ancient and sound tradition that young men and women go to a university to become all-round citizens and not merely to learn a special skill. Likewise, the Committee believes that no distinction should be drawn between men and women students: in the national interest all potential talent should be given its chance to develop. Full-time paid employment is not the only means of enriching the national life, and a sustained increase in the number of highly educated mothers, as well as fathers, would benefit the nation to an extent that is not yet generally appreciated.

The Committee recognizes that its proposals can scarcely avoid having an effect on both schools and universities, though this is not intended; and it thinks that the greater responsibility should properly rest on the universities and colleges. Nevertheless, it

* Ministry of Education: Scottish Education Department. Grants to Students: Report of the Committee Appointed by the Minister of Education and the Secretary of State for Scotland in June 1958. Pp. xii+135. (Cmnd. 1051.) (London: H.M. Stationery Office, 1960.) 6s. 6d. net.

is impressed with the need for uniformity in the treatment of applicants for awards, and the Standing Advisory Committee it proposes is intended to assist here and to deal with the practical and human problems that will inevitably present themselves. No feature of the report is more impressive than the way in which the Committee recognizes these human problems and seeks to dispel the anxiety which many students at present inevitably experience while waiting to hear whether they will receive the grant on which their ability to take up a university place depends. This is very notable in the comparatively minor recommendations contained in the final chapter of the report, which discusses various practical matters of importance to students and their parents.

Nor is this human interest one-sided. In commenting on the importance of interviews, the Committee expresses the hope that universities, in addition to making more uniform the present procedures and conditions of admission, will extend so far as possible the practice of interviewing candidates before acceptance. Quite apart from the function of interviews in helping to determine the choice between one border-line candidate and another, it believes that the influence of a properly conducted interview on the student himself is important. As the Committee rightly remarks, the student will in effect be applying for a grant of a large sum of public money, and it is unquestionably right that a positive effort should be made, in an interview, to remind the applicant that he or she is seeking the privilege of being backed by the community. Recognition that a university place is a privilege—although it has been earned—is too often overlooked, and if it were more generally remembered, as well as its implications, the criticism that many graduates look on a post as something which the community owes them, rather than as an opportunity to serve the community, might be less frequently heard.

This could be the more important if universities became in effect free for all as a result of the adoption of the majority recommendation to abolish the parental contribution. The Committee's observation, moreover, gains strength from the obvious liberality of the views of its members and their sympathetic understanding of the point of view of the student. The Committee is emphatic that students throughout Great Britain should have the freedom of choice of institution. Much of the value of higher education, it points out, lies in the contacts the student makes and the life he leads within the student community and outside the lecture room and the laboratory; and to get the full benefit, it is important that the student body should not be drawn from too narrow a field—it will gain richness from a wider field. Again, the Committee believes that it is important that students should be able to rely on a grant for the full normal period of the course on which they have embarked, and it specifically recommends that all awards should be so made, subject only to satisfactory progress and conduct and to appropriate measures to deal with arbitrary changes of courses by indifferent students while taking care of reasonable changes, particularly at the end of the first year.

Of the selection for awards no more need be added than that the Committee is emphatic that the universities themselves are best fitted to select those who should receive a university education. It thinks that since effective selection by local education authorities is mainly confined to border-line candidates, the return, in terms of improved selection and of public money saved, is small, and the present arrangements impose an undue burden on the schools and their pupils. It also concludes that no second process of academic selection should be required for the main stream of entrants to first-degree and comparable courses.

The major issues on which the Committee was agreed may be briefly reviewed. It recommends in the first place that the method of expressing the maximum grant in terms of "payment of fees plus a standard sum for maintenance" should apply throughout Great Britain, and each standard figure should include the same amount for books, clothes and incidental expenses. It rejects firmly the suggestion that the grant for books should be in kind. The student should be treated as a responsible person and left free to spend the money granted according to his own circumstances and his judgment of his own needs. Further, it thinks it important that the student and his university teachers should be aware how much has been allowed in his grant for the purchase of books, and accordingly publication of this amount is recommended. It also recommends that a fixed sum for travelling expenses should be included in the standard figure of maintenance, and it endorses the principle of grouping universities and of including in the standard figure of maintenance for each group an amount for the maintenance of students living in colleges or halls of residence or in lodgings. Holders of awards should be covered for the payment of national insurance.

In all this detailed assessment of the needs of holders of awards, the Committee treats the student as a responsible person, and if its sensible recommendations are adopted, it is only reasonable to expect that this will be generally recognized by students themselves. The recommendations for grants for vacations and vacation courses are no less sensible, and are generous although clearly recognizing the public interest. They are based on five principles: that award-holders should receive standard grants for maintenance throughout the vacations; this grant should be calculated at the same rate as that for 'keep at home' during term-time; the grants should be paid before the vacations for which they are given; deductions from subsequent grants should be made for any vacation periods in which the holder has been in paid employment; the standard vacation grant for the final long vacation should be calculated to cover four weeks only, and should not be subject to any declaration about paid employment.

The miscellaneous recommendations stress the importance of dealing with all applications for awards

as quickly as possible. A pamphlet should be printed to provide a general guide to the awards system, and if university and comparable awards remain the responsibility of local authorities, it is essential that full information about the local arrangements is widely available in each area. When the student is notified of the amount of his grant, he should also be told the conditions attached to the award and given a statement of the way in which it will be administered. If parental contributions are retained, the parent should be notified both of the amount of the grant to the student and of the amount of the parent's contribution. The recommendations regarding payment of awards are also sensible and would eliminate the practice of withholding payments until a certificate of attendance has been obtained from the university or college authorities. Instead, subject to the agrement of the university or college authorities, cheques for ordinary grant payments should be made available to award-holders through the university or college office, on the lines now followed by the Ministry of Education for State scholars. In addition, the Committee recommends that all award-holders should be able, on application, to draw an advance payment of up to £30 before the start of their first term.

Collectively, these minor recommendations should not only simplify the present system and establish some uniformity of practice, but also remove some of the anomalies and grievances which have been a source of complaint and even hardship to students. Nor is the cost excessive. With the abolition of parental contributions, the net cost of which in 1958–59 is estimated at £5·5 million, the total increased cost arising from the proposals of the report is about £8·2 million, compared with £7·1 million if the modified parental contributions suggested as alternative are adopted. For a university population of 135,000, assuming the students from the United Kingdom following first-degree courses continue to form roughly the same proportion as in 1958–59, the cost to public funds is estimated at £22·9 million under the present system, £32·3 million with modified parental contribution and £33·8 million if the parents' contributions are abolished.

Financial considerations should thus not be of decisive importance in determining the abolition or retention of the parents' contribution, particularly in view of the general agreement that some modification is required; and the difference in opinion between the advocates of a more generous system and those who would abolish the parents' contribution does not turn on cost. The evidence submitted to the Committee indicated that, while none of the bodies giving evidence was entirely satisfied with the present system, the reforms suggested differed widely. Out of seventy organizations, forty recommended complete abolition of the parents' contribution and fifteen its abolition for approved fees while retaining it for maintenance. Moreover, it was apparent that differences of opinion existed within the organizations themselves, and the division of opinion on the Anderson Committee itself thus reflects that existing in Britain generally.

Nevertheless, it is important to note that there was a considerable measure of agreement on the Anderson Committee. It was agreed, for example, that none of the half-measures suggested offers a satisfactory solution, and that a straight choice has to be made between relieving parents altogether or maintaining a system of assessment of parental income for awards purposes. Again, the Committee agreed that the abolition of parental contributions would be a fundamental change, and that an assessment of its probable effects is a matter of judgment. In the Committee, the difference of opinion lies in the assessment of those effects. The majority is firmly convinced that they would be beneficial, and the argument is cogently and convincingly presented. The minority sees a strong possibility of harm, and does not regard the difficulties of the present system as serious enough to warrant running the risks involved in abolition; but that case is far less convincingly argued. None the less, all are agreed that the demand for parental contribution must deter some well-qualified boys and girls from entering universities, that the present system imposes too heavy a burden on many parents and students, and that if parental contributions are retained they must be substantially reduced. There is also agreement that the level of contributions proposed in the report, which would, for example, relieve a married couple with one child from any contribution if their gross income is less than about £1,000, would largely remove the hardship on the middle-income parent on whom the present scale bears most heavily.

Having gone so far, the majority of the Committee would complete the process by abolishing the parental contribution altogether; believing that the higher the income below which no contribution is expected, the greater the number of families who get complete exemption and the smaller the actual yield both absolutely and in proportion to the cost and the administrative effort involved in collection. Secondly, and even more important, the smaller the number of families who are still required to make a contribution, the less the exaction of that contribution can be justified on grounds of general equity and the greater its resemblance to a discriminatory tax on a limited section of the community. If the level of contributions recommended in the report is adopted, about 60 per cent of all the present university and comparable awards would not be subject to parental contribution, and there is logic in the Committee's contention that a system of university awards should not superimpose what is in effect a second taxation system on some parents.

Whether or not the argument that abolition of the parents' contribution would provide a great stimulus to higher education is sound, the elimination of administrative complexities is undoubtedly an advantage, and it is clearly unjust and illogical to allow an awards system to penalize a small class of parents; nor does the minority advance any convincing social or practical reasons for its retention. Prof. Brinley

Thomas, who also considers that the Committee does less than justice to the role of the State scholarship in the educational system of Britain in recommending that all types of State scholarship should be discontinued and suggesting that the State scholarship should be retained in a new form, with possibly a new name, dissents from his colleagues who wish to retain the parents' contribution in advocating at the same time the introduction of substantial sixth-form bursaries in the maintained schools. He regards public expenditure on ensuring that gifted boys and girls in the public sector are able to stay at school until the age for entry into the university as a first priority, and holds that to remove or reduce drastically the cost of university education for middle-class families while doing nothing to stop the leakage of talent among children at the age of fifteen in the lower-income groups would be an unwise and unjust use of public funds.

Prof. Brinley Thomas's note of reservation is much more cogently and convincingly argued than his colleagues' case for retaining the parental contribution; but nevertheless, he does not meet the real objection, namely, that to retain that contribution under present conditions is tantamount to differential taxation. It should be remembered, moreover, that the report proposes that parents should no longer be able to deduct child allowances from their taxable incomes in respect of children receiving grants. While it is perhaps understandable that the report should be somewhat hesitant in dealing with the financial aspect of the administration of grants as between the Government and the local authorities, the Committee leaves the Government in no doubt as to where the weaknesses of local authority administration lie and the need for some central direction in order to secure uniformity. That question must be settled by the Government before effect can be given to the rest of the report, and it is to be hoped that there will be no undue delay in reaching a firm decision and giving effect to some such simpler and fairer system of awards as the report outlines and removing injustices which were recognized as such by all its members.

UNIVERSITIES OF THE COMMONWEALTH

Commonwealth Universities Yearbook, 1960

Edited by J. F. Foster, assisted by T. Craig. Thirty-seventh edition. Pp. xxxi+1509. (London: Association of Universities of the British Commonwealth, 1960.) 84*s.*; 13 dollars.

THE bulky appearance of this well-established Year-Book is an effective reminder of the extent of higher education in the Commonwealth. It was first launched in 1914, and, because of stable conditions in its first year of life, did not need revising each year.

Since then thirty-seven volumes have appeared, and, because of rapid changes, now needs to be reissued in new dress each year.

When the Year-Book edition of twenty years ago was published, it included references to eighty separate university institutions and recorded a (full-time) population of less than a quarter of a million students. In the present volume 135 institutions are encompassed with a total student population of 1,115,000.

The 1960 Year-Book follows a year of remarkable progress in Commonwealth educational development made noteworthy by the Commonwealth Education Conference held at Oxford in July, 1959. One of the recommendations of the Conference was the launching of a scheme to award a thousand scholarships, mainly at the postgraduate level, tenable in the 'sponsoring' countries by students from the rest of the Commonwealth. A special appendix describes the working of the scheme.

Five new universities are included for the first time. These are Monash University in Australia, and Gorakhpur, Jabalpur, Marathwada and Vikram Universities in India; their inclusion is itself tribute to the way the Commonwealth countries are developing their professional man-power. One small item which will give much pleasure in Britain is that "the youngest member of the university family is the University College of Sussex at Brighton which was formally constituted in 1959 . . . . It is hoped that enough progress will have been made with academic buildings and the recruitment of staff for 200 students to be admitted in October, 1962".

The format of the book follows the pattern of earlier years, universities being grouped in countries, and each group is introduced by an essay describing in general terms the university system of the country and its general provision for higher education. For the first time the British section includes a table showing at which colleges of the University of London courses for a first degree are provided in fifty different subjects of study. This supplements a similar table for the universities of the United Kingdom as a whole which was warmly approved when it was introduced two years ago; it re-appears in the present edition.

One feature of the book which should bring legitimate pride to graduates from universities of the 'Mother Country' is that there are few faculties of universities in member countries which do not include some members of staff with British degrees. Before becoming overwhelmed with self-gratification, however, British graduates should study the interesting faculty developments in other Commonwealth universities and reflect whether or not development in Britain is still too hide-bound by tradition.

The value of this tremendous compilation is well brought out in Appendix 1. Without introduction or explanation it includes details of the Universities of the Republic of Ireland; it is encouraging to find a way of seeing that our Irish friends are not left out.

Other appendixes deal with university admission requirements, particulars of students from other countries who are not at present in British universities; details of British academic institutions abroad and of the Association of Universities of the British Commonwealth whose book this is. Could they now be persuaded to ask a research student to investigate the entrance examinations to all Commonwealth universities, the patterns (and costs) of administration, student admissions, rejections and achievements, and to issue a report? The overall view would be received with interest.

FIRST CENTURY ROMAN ARCHITECTURE

Roman Construction in Italy from Tiberius through the Flavians

By Marion Elizabeth Blake. Carnegie Institution of Washington. Publication No. 616. Pp. xvii+195+13 plates. (Washington, D.C.: Carnegie Institution of Washington, 1959.) Paper 8.25 dollars; cloth 9 dollars.

IN 1957, Dr. Marion Blake published her "Ancient Roman Construction in Italy from the Prehistoric Period to Augustus", a chronological study of the methods and materials employed by the earliest Roman builders which has proved of great value to scholars. Now she continues her researches through the first century A.D. to the end of the Flavian period, incorporating into them, as before, many of the copious notes and photographs left by the late Dr. Esther Van Deman for a work of this kind. The extensive bibliography shows, however, how these have been supplemented by the work of other scholars, while the illustrations include excellent photographs by Prof. Lugli and Dr. Ernest Nash.

About half the length of its predecessor, the new volume begins with a brief introduction describing the building materials in use during the time of Augustus and the types of evidence valuable for chronology. After this the detailed examination of the evidence begins in Chapter 2, covering the Julio–Claudian Epoch from Tiberius's accession in A.D. 14 to Nero's death in A.D. 68. Tiberius seems to have adopted no innovations, apart from an increased use of brick-faced concrete, and Claudius built comparatively little in Rome. But Nero was anxious to beautify and enliven his city, and in the early years of his reign new baths and an amphitheatre arose as well as a palace, the Domus Transitoria. The great fire of A.D. 64 gave him an opportunity for town-planning which he used wisely, replacing the narrow, crooked lanes with wider streets and open spaces. Less timber was used for building and the employment of Gabine stone and peperino was encouraged as these materials were believed to be more resistant to fire. Plans were made for the great Domus Aurea which replaced the Domus Transitoria, but the new palace was never completed, and many interesting constructional details are revealed by its unfinished state. The walls show the first use of concrete faced with the triangular bricks made by cutting square tiles diagonally in half, and several new developments in vaulting are also noted. Roman private houses and tombs of this period are also briefly described, and some consideration is given to building in other parts of Italy, notably at Ostia and at Pompeii, where the earthquake of A.D. 62 made extensive reconstruction necessary.

The third chapter takes us from the Civil War of A.D. 69 to Domitian's death in A.D. 96. Vespasian, the first of the Flavian emperors, continued Nero's policy of reconstruction. He also initiated the building of the Colosseum, work which was carried on by his sons, Titus and Domitian. Dr. Blake's account of it is full of details of human as well as of structural interest; for example, the use of awnings to shade the spectators from the sun, and the various barriers and other measures needed to protect them from the wild beasts. Titus's brief reign is chiefly noteworthy for another disastrous fire in A.D. 80. As a result of it his successor, Domitian, was faced with problems similar to those which confronted Nero and he also seized the chance to add, enlarge or elaborate. Temples, baths and fora, as well as the great palace on the Palatine hill, were all under construction during his reign. The chapter concludes with an interesting description of buildings on the imperial estates, various engineering works and public monuments in other parts of Italy, and the latest developments at Pompeii and Herculaneum prior to Vesuvius's eruption.

The final summary includes a brief résumé of building methods, and shows us how the development of a nearly perfect concrete left architects free to experiment with ground-plans of increasing elaboration. Not all the problems of weight and stress, however, had yet been solved, and consequently much of Domitian's vaulting was to need reinforcement in Hadrianic times. This carries us beyond the limits of the present book, and we look forward to the next volume in the series which, Dr. Blake promises us, will carry her researches to the time of Constantine.

JOAN LIVERSIDGE

THE MECHANISM OF ADAPTIVE BEHAVIOUR

Design for a Brain

The Origin of Adaptive Behaviour. By W. Ross Ashby. Second edition, revised. Pp. ix+286. (London: Chapman and Hall, Ltd., 1960.) 42*s*. net.

AT one time it was not generally thought that learning could be regarded as a mechanical process. The view that it could is now widely held. This is due partly, if not largely, to Dr. Ashby's writings over the past twenty years. A major work of his, "Design for a Brain", now appears in its second edition. This is virtually a new book. Its object is to analyse the character of the activity of the brain, and learning in particular, in mechanistic terms. To drive home this approach an analogue computing device, the homeostat, has been so constructed as to exhibit purposeful, adaptive behaviour. The description and discussion of this device remains one of the central features of the book.

In broader terms, "Design for a Brain" is devoted to the consideration of goal-seeking mechanisms that change continuously. Mechanisms using discrete-step operations are easier to analyse, but continuously changing mechanisms are more readily comparable with organisms. Dr. Ashby is a creative thinker and a rigorous one. He defines precisely all the terms he uses. He deals with dynamic systems, that is, with sets of variables selected from vast numbers of variables that constitute machines. The systems considered are those that are stable and that show purposeful behaviour. The stability in a system, brought about by feedback, characterizes only certain combinations of the parts of the system. It is contended that adaptive behaviour of a system, or learning, is mediated by two feedback loops, one forming a close link between sensory and motor channels and another "working intermittently and at a much slower order of speed". Such a system is called ultra-stable, and the behaviour of the homeostat exhibits ultra-stability.

The action of the brain appears to be in principle like that of an ultra-stable system, but, of course, it is much more refined than anything so far designed.

Chapters 1–9 of the book are concerned with the basic problem of the mechanism of goal-seeking, adaptive behaviour, while Chapters 10–18 give consideration to the refinements of such a mechanism. One of them is a method for coping in an efficient manner with recurrent disturbances in the environment. Again, the homeostat is so constructed that every unit interacts with every other unit. It is shown that this makes for slowness of adaptation. It is known that in the brain not all units are interconnected. The book considers designs in which units are not fully joined, and where success at learning that is partial is not disregarded. This brings us a little nearer to biological reality which the machine design attempts to meet. Another step towards this end that is being considered is poly-stability; a poly-stable system "is any system whose parts have many equilibria and that has been formed by taking parts at random and joining them at random".

Dr. Ashby is knowledgeable in many fields. The reader cannot fail to be impressed by the precision of his exposition. Even so, many will retain a sense of doubt. Put briefly, the doubt is as to whether the approach represented by "Design for a Brain" is the right one towards a better understanding of the functioning of the brain. In striving towards this aim there is, of course, no substitute for experimental studies in physiology and psychology. The cybernetic approach is likely, however, to be of some help to many of those who are engaged in experimental work.

W. Sluckin

SURVIVAL IN THE COLD

Recent Research in Freezing and Drying

Edited by Dr. A. S. Parkes and Dr. Audrey U. Smith. Pp. vii+320. (Oxford: Blackwell Scientific Publications; Springfield, Ill.: Charles C. Thomas, 1960.) 63*s*. net.

In 1951 the first international symposium on freezing and drying created a great deal of interest and the Institute of Biology, which organized it, decided that so many advances had been made that a second symposium was justified in 1958. Unfortunately, it has taken two years for publication of the papers presented so that the volume is already out of date in many of the subjects with which it deals. At the first symposium Fry had shown that the addition of glucose decreased the lethal effect of freeze-drying for micro-organisms, and many of the papers read in 1958 described work or theories to explain the role of adjuvants such as glucose, sucrose and glutamate. The effects varied with the method of freeze-drying used; Greaves showed that a mixture of 5 per cent dextran and 5 per cent sodium glutamate was excellent for bacteria that were pre-frozen but was not suitable for the spin-freeze method of drying; for this method a mixture of 5 per cent bovine albumin and 5 per cent sodium glutamate was satisfactory. Obayashi reported that for *BCG* vaccine glutamic acid alone gave better survival-rates than glutamic acid plus sugar. On the other hand, Muggleton used a mixture of dextran, sucrose and glutamate for his *BCG* vaccine. W. J. Scott believed that certain essential metabolites of living cells may be toxic to dried cells. This hypothesis is controversial, but Scott's finding that survival depends on the water activity of the dried product will find more general acceptance. G. R. Scott and Rampton sought guidance on the influence of size of batches in the preparation of virus vaccine. Their experiments suggested that the difference in antigenicity between different batches was much less than Daubney had postulated in 1951, and asked if they had misinterpreted their results.

An important part of the symposium, and so of the book, dealt with the freezing and drying of tissues, and here the greater advances were recorded. The use of glycerol to prevent ice crystal formation has done much to make the freezing of cells a safe procedure but, of course, glycerinated material cannot be dried. Bone, skin, arteries and corneas have all been freeze-dried; but, as Parkes says, the tissue cells seldom survive, and the material only forms a scaffolding on which new tissue can form. In the cornea freeze-drying modifies the protein constituents, and the antigenicity of the material is so reduced that it is possible to use tissue from another animal species.

Several papers dealt with the theoretical aspects of freezing and drying and these have a special interest for the biophysicist. In general, the papers are printed as they were delivered, and, as few make attractive reading, it is a pity that the editing was not more strict.

S. T. Cowan

MATHEMATICAL METHODS IN ECONOMICS

Studies in Linear and Non-Linear Programming

By Prof. Kenneth J. Arrow, Prof. L. Hurwicz and Hirofumi Uzawa. With contributions by Hollis B. Chenery, Selmer M. Johnson, Samuel Karlin, Thomas Marschak and Robert M. Solow. (Stanford Mathematical Studies in the Social Sciences, Vol. 2.) Pp. ix+230. (Stanford, Calif.: Stanford University Press; London: Oxford University Press, 1958.) 60*s*. net.

This book is the second in the series, Stanford Mathematical Studies in the Social Sciences. It is a collection of research papers, and most of the results were obtained during 1955–58. It is divided into three parts, after an introductory chapter which explains and summarizes the contents of the various papers.

The first part is purely mathematical and deals with existence theorems, including the Kuhn–Tucker theorem on concave programming. There is a paper by Hurwicz on programming in linear spaces, and this includes an account of linear topological spaces and their properties. This account is of an introductory nature and will help considerably the reader who is not already familiar with the field.

The second part is concerned with the gradient method, so called because the gradient of a certain vector figures prominently in it. The idea is to solve a certain set of differential equations by using the finite difference approximations. The gradient method is available for non-linear programming problems, but for a linear problem it is not suitable since it gives rise to an oscillatory rather than a convergent path.

The third part deals with methods for solving certain particular problems, linear or non-linear. One problem concerns price speculation, where an individual may buy or sell a commodity, but is not allowed to borrow. If the costs are those for holding a stock

of the commodity the problem is linear; but if the costs are brokerage fees proportional to the amount bought or sold the problem is non-linear. Optimal operating policies are determined in each case. Another problem deals with the allocation of n tasks to m machines, where the machines are ordered in terms of increasing capability and the tasks in order of increasing difficulty. A third problem—directly in the field of inter-industry economics—is solved by a method which was originally applied to the problem of resource allocation in under-developed countries.

Mathematics has by now invaded in force the social sciences. The vigour with which various classes of problems in economics, for example, are being attacked mathematically is quite surprising. This situation implies an increasing divergence between what one might call the verbal or linguistic economist and the econometrician, who deals, so far as possible, with measurable concepts. The book under review is certainly meant for the latter—in fact it will probably only be understood by the mathematician interested in economics rather than the economist who would like to understand the workings of mathematics within his subject.

L. S. GODDARD

MANAGEMENT IN THE UNITED STATES AND THE U.S.S.R.

The Red Executive

A Study of the Organization Man in Russian Industry. By Dr. David Granick. Pp. 334. (London: Macmillan and Co., Ltd., 1960.) 21*s*. net.

AN amalgam of wide reading, many personal discussions and actual visits to a variety of industries inside the U.S.S.R. and the United States has enabled Dr. D. Granick of the University of Wisconsin to provide a great deal of comparative information about Russian and American management which hitherto has been subject to little more than speculation.

During his investigations Granick set out to determine the educational background of the managers, the pressures and incentives which govern their lives, the tasks before them and how the factories and social environment of the U.S.S.R. differ from those in the United States. For the British student, at least, the book is most satisfying and worthy of recommendation to many readers. The most striking of the findings are those which show that the similarities between the Russian and American management greatly outnumber the differences and, indeed, link them more closely than American and British managers.

The main differences stem from the nature of each country's economy. In the U.S.S.R. the marketing manager does not exist; everything produced can be sold. Salesmanship, advertising and product design to meet competition and make last year's model obsolete, which are major American pastimes, do not exist in the U.S.S.R. Their problem is the getting and allocation of raw material, and the American purchasing officer is replaced by a procurement official.

A major difference might appear to be the place of the trade unions. Whereas in the United States the unions are reputed for their ability to drive hard bargains and, sometimes, disrupt production, in the U.S.S.R. the trade unions are imagined to be apostles of productivity and essential props for management. This may well be, but those familiar with the politically backed works commune would support Granick's statement that the American manager has little cause for grumbling compared with the Russian. A surprising difference is that the Russian manager is much more prepared to try out new ideas than his American counterpart. Granick believes that this may spring from the much more "theoretic" nature of the Russian training.

Among the many similarities of the Russian and American managers, the most striking (and from the British point of view the most challenging) lies in their educational backgrounds. In the U.S.S.R. the majority of managers get full-time education up to university level; in the United States little more than half are college-trained. In the U.S.S.R. the majority have engineering degrees; in the United States almost half the college-trained managers have general arts degrees. The American preoccupation for the "rounded" man is bound up with the fact that students are free to choose courses for themselves. This is also true of the U.S.S.R., but the courses made available are circumscribed by the needs of the economy.

The American graduate business training schools are largely missing in the U.S.S.R., which professedly believes in the value of manual work in introducing graduates to industry. The differences are not so great, however, because the Russian engineering graduates take a postgraduate diploma which necessitates involvement in an industrial organization. Another point of similarity is that, although the position is hardening in the United States, in both countries the way to the top is still wide open to the boy of ability and application.

Many other matters emerge to make this book of absorbing interest. Russian academic authorities, for example, appear to be as hazy about Russian management practice as most American university lecturers are about what goes on in their business training schools. The echo from Britain would also resound to complaints from research workers from both sides of the Iron Curtain about the difficulties they meet in obtaining permission to study factory administration in any kind of depth.

In both countries students are encouraged to work in industry as part of their educational (*sic*) development. One large American university has about one-seventh of its better-off full-time students doing forty-hour-a-week-jobs. In the U.S.S.R., as in Britain, the student is expected to study full-time during university term. Before graduating to the managerial level, however, the Russian spends a considerable period as foreman or assistant. The attitude of some British university teachers to vacation work for students may need reconsideration.

Granick also deals with the size of industry in both countries, the inverse operation of Parkinson's Law in the U.S.S.R. over the past twenty years (this supports those who believe it to be based on false premises), the lack of definition of the foreman's job in the United States, U.S.S.R., and Britain, and enough related matters to make his book worth a place in libraries in many countries. He should now perform a service to British management by preparing a second volume describing the observations he has made during his year as a Fulbright Fellow in Great Britain.

T. H. HAWKINS

The Road to Man

By Herbert Wendt. Translated by Helen Sebba. Pp. 253+31 plates. (London: Lutterworth Press, 1960.) 30*s*. net.

IN his preface the author of this work confesses "'The Road to Man' is therefore not just a sober, factual account of the animal world, but a kind of novel of the animal world, a novel with many chapters—which are only superficially disjointed". Depending on one's estimate of superficiality, this is an acceptable description of the book. In the course of twenty-nine brief chapters reference is made to virtually the whole of the animal kingdom. It follows that scant justice is accorded to most aspects of the subject, and the general reader, for whom the book is intended, will close it with some dissatisfaction at having been brought to innumerable doorways and then led hastily away in order to complete his tour before closing time. Mr. Wendt's objective has been to demonstrate man as one among many animals, and not, as many would like to think him, the central figure about which the rest of Nature revolves. It cannot be said that he has successfully achieved this admirable end, although certainly he has demonstrated an enormous diversity of natural phenomena. But the pattern inherent in his sub-title, "An Informal History of Evolution", is sacrificed to a disjointed series of examinations of individual threads in the weave.

Inevitably, with so much ground to cover, the author has taken many short cuts to what he believes to be the truth, and many of these are tolerable approximations to probability. But it is impossible to pass unchallenged such statements as "Protoplasm which has the same chemical structure as that of the sea at the time when life is believed to have evolved" (p. 22), or "A lot still had to be accomplished before the lampreys became real vertebrates" (p. 64). Nor can one accept the assumption that "the mature eel being totally oriented to seaweed and deep sea . . ." is thereby constrained to migrate to the Sargasso Sea in order to breed; while the description of the actively carnivorous habits of sea urchins can only be ascribed to the author's aforementioned fictional approach to his subject. The book is embellished with a number of excellent photographs, rather more randomly selected than is the text. A word must be added in praise of the translation, the style of which is excellent. L. A. Harvey

Studies on Agar Gel Electrophoresis

Techniques—Applications. By R. J. Wieme. (University of Ghent: Department of Internal Medicine.) Pp. 531. (Brussels: Arscia Uitgaven, N.V., 1959.) 680 Belgian francs.

THE beautiful technique of immuno electrophoresis introduced by Grabar and his associates gave great encouragement to the choice of agar gel as a supporting medium in zone electrophoresis. It has a number of advantageous features such as good resolving power for proteins, adaptability for very small scale work, and its ability to be converted to a thin transparent film after electrophoresis and staining makes it suitable for photometric scanning. These and other features are discussed in much detail by Dr. Wieme in his monograph. The author describes his various modifications of carrying out the electrophoretic run on microscope slides coated with agar, and of interest is an ultra-microprocedure whereby as little as 0·03 μl. of serum or 2 mgm. of tissue may be analysed. Applications, mainly of one particular micro technique to studies of constituents of normal human sera and of animal tissue enzymes, are described, and thereafter clinical studies of sera in some fields of wide interest, namely, plasmocytoma and primary macroglobulinæmia, modification of γ-globulin in disease and examination of lactate dehydrogenase in disease. All the sections include a thorough survey of the work of others and a good coverage of the literature on the various subjects discussed.

This monograph gives a highly detailed exposition of practical and theoretical aspects and is therefore of value to those interested in agar-gel electrophoresis. Unfortunately, it is written as a thesis and therefore not entirely suitable for general reading. It could form the basis of a very useful book of wide appeal if the subject-matter were better arranged, unnecessary details cut out and the English improved. (Some of the phrases, being literal translations of Flemish idiomatic expressions, are very quaint, and would delight the heart of Paul Jennings.) There is a great deal of useful information in this work, and so it would be a pity to leave it in its present form and not attempt to make it more presentable and more reasonably priced. R. Consden

The Indian Ephemeris and Nautical Almanac for the Year 1961

Pp. xxvi+448. (Delhi: Manager of Publications, 1960.) Rs. 12.50 nP.; 19*s*. 6*d*.

THIS volume, the fourth annual issue of the "Indian Ephemeris", plays an important part in the development of modern astronomy in India. It is a worthy tribute to the enthusiasm and persistence of the late Prof. M. N. Saha, to whom the original proposal and a large part of the preliminary organization and planning were due.

The initial impetus for the introduction of a separate "Ephemeris" arose from the necessity to reform and unify the many different calendarial systems in use in India. It is therefore not surprising that no fewer than 48 pages are devoted to the Indian Calendar, giving full details of the Reformed Calendar of India.

In other respects the ephemeris follows very closely the models adopted by the other national ephemerides. The fundamental calculations have for long been shared between the ephemeris offices of France, Germany, Spain, the United Kingdom, the United States, and the U.S.S.R.; and these have been made readily available for the preparation of this volume. The work of preparation has thus largely been concerned with compilation, presentation and printing: these have been done excellently under the competent guidance and supervision of Sri N. C. Lahiri. But the ephemeris of the Moon at ephemeris transit, and the special tables of sunrise, sunset, moonrise, and moonset for India have been calculated specially.

The low standard of practical astronomical knowledge in India, coupled with the prevalence of astrology, was one of the main reasons for the inauguration of a new national ephemeris at the time when, in other countries, there was a strong tendency towards unification. The introduction of the "Indian Ephemeris" has already started to inspire serious astronomical work in India; in general competence and in attention to detail, of both content and presentation, no better standard for that work could have been set up. D. H. Sadler

WORLD FOOD AND POPULATION

IN recent years the British Association for the Advancement of Science has become increasingly aware of the necessity to re-align its activities to meet modern needs. Gone are the days when its annual meeting provided a forum for discoveries, great and small and made within the preceding twelve months, to be disclosed to an expectant world: radio, television and the scientific and technical press are only too anxious to publicize at once whatever discoveries are deemed to have 'news value'. But not only is the lay public bewildered by this bombardment; scientists themselves also find it increasingly difficult to assess the meaning and implications of work other than that in their own, often narrow, specialisms. The annual meetings of the Association have always provided a welcome opportunity for scientists in the many branches to meet one another and to make contact with an interested public. Now the Association is making a determined effort to present a balanced view of scientific progress to a wider audience—through its work with young people, its programmes of lectures at local centres, the new and attractive format of its bi-monthly journal, *The Advancement of Science*, and in other ways. At the Cardiff meeting a bold new experiment was tried. Coincident with the inauguration on July 1 of the five-year Freedom from Hunger campaign of the Food and Agriculture Organization of the United Nations, the Association chose as a central theme, to be emphasized by as many sections as wished, the numerous aspects of population pressure on land resources. A complete innovation was the suspension of all sectional programmes for one whole day—Monday, September 5—in favour of two plenary sessions of the entire Association in the Sophia Gardens Pavilion, devoted to a symposium on "World Food and Population".

Some 2,000 members assembled to hear the president, Sir George Thomson, open the proceedings. He reminded the audience that it was the success of medical science that necessitated a falling birth-rate to match the falling death-rate. It was not right that scientists should pass the problem to the politicians who, in any event, had to deal with the toughest and most resistant material in the world, human nature.

Dr. Norman C. Wright, deputy-director general of the Food and Agriculture Organization, presented the facts of the world food situation as they are known to that Organization. He drew a distinction between under-nutrition and malnutrition, and gave reasons for using calorie intake as a measure of under-nourishment. What proportion of the world's people can be described as under-nourished clearly depends on the standard taken. While British conditions suggest that 2,800 calories a day are necessary for the development of full health and vigour, requirements for the peoples of tropical climates, with lower average body-weights and stature, are clearly less. But 60 per cent of the world's population appear to have an intake of less than 2,200 calories, of which nearly half have less than 2,000 calories a day. With a poor harvest, under-nutrition passes very easily into semi-starvation and loss of mental alertness, and even moral standards. Malnutrition accompanies under-nutrition, but is also found when the intake, though adequate in amount, is derived from an insufficiently varied diet—especially a shortage of protein from an all-cereal diet. Such malnutrition is reflected in the presence of several deficiency diseases—notably kwashiorkor—liable to affect children after weaning, that is, when they are transferred from a milk to a cereal diet. Dr. Wright urged that neither distribution of food surpluses from countries such as the United States nor a world-wide adoption of vegetarian diets could solve the problem—only the widespread and rapid application of recent scientific and technical advances.

Sir Alexander Fleck took up the latter point in the afternoon. Apart from advances in all kinds of techniques constantly being made, there is an adequate body of knowledge, and has been for many years, to secure sufficient food, indeed abundant food, for all mankind if only that knowledge is applied. There are six ways of producing more food: by extending the area under cultivation, substituting more suitable crops or varieties and animals or breeds, providing more water, more nutrients, adopting better techniques, and increasing efficiency of crop or animal use. A comparison of present-day yields, for example, between Japan and India in rice shows at once the enormous possibilities to be achieved by providing crops with adequate nutrients and water.

Both Sir Alexander and Prof. H. D. Kay—obviously through his extensive practical experience in recent years in many countries—stressed the human aspects of the problem: how to get right to the peasant cultivator to help him to help himself. The missionary spirit and the sense of dedication to a cause is nothing new to the British: both are badly needed to-day. Equally vital is the need to convert governments and administrations to the necessity for constructive agricultural policies. Too often agriculture is a Cinderella among government departments.

Prof. David Glass, speaking on the present trends of world population, stressed the double importance of focusing attention on the remainder of the present century, since by 2000 A.D. world population is most likely to increase to more than 6,000,000,000. Because of the present age-composition of the population in the less-developed countries, even such sharp reductions in fertility as might result from a rapid spread of birth control would not prevent very considerable increase in numbers by the end of the century. Even with a 50 per cent fall in live births in India during 1956–81, the population would still increase by more than 200 million by 1986. At present the evidence does not justify an assumption of a general fall in fertility: it is more realistic to plan for an increase of world population at the rate of at least 1·6 per cent per annum. Prof. Glass considered that we must be prepared for a very massive underpinning of planned economic and social development. Although he included emigration among the measures to be included, he noted that the present increase in world population in one year is about equal to the

net oversea migration from Europe in the 150 years 1800–1950. He noted, however, the symbolic value of emigration in promoting also economic and social change. In view of the relative ease of increasing food output in Western lands in mid-latitudes, the planned disposal of food surpluses in the West may be no less essential than the raising of agricultural productivity in less-developed regions.

Prof. Arthur Lewis, recently elected to the office of principal of the University College of the West Indies, discussed economic conditions for greater output under the four main headings of incentives, knowledge, capital and organization. He placed expanding demand first among incentives: rising standards of living in the towns demanding more food and more expensive foods. Provided the fruits of the farmer's labours accrue to him and not to the middle man or landlord, he doubted the importance of price stability, preferring freedom of prices to rise. Prof. Lewis was insistent on the enormous capital requirements needed in farming, but some of his assertions regarding organization can be questioned. In his own summary he asserted, "in most of Asia, where labour is abundant, mechanization contributes only where it brings under the plough land which could not otherwise be cultivated, or makes possible more crops in a year. Mechanization has more to contribute to the under-populated parts of Africa". He advocated subdivision of large properties, especially in Latin America, and considered that compulsion should be used when farmers are too ignorant and conservative to use better seeds or improved cultural practices, and advocated collectivization where necessary. As a West Indian who has a wide experience in many lands, it is interesting that Prof. Lewis more than any other speaker should advocate such forceful methods.

Prof. Dudley Stamp, speaking on land use and food production, stressed the contrast between a population not only increasing at an unprecedented rate—in excess of 45,000,000 a year—but also with rising standards of living, which meant both more and more varied food, more spacious layout of homes, facilities for recreation and movement and other extensive demands on land, with the known, fixed and broadly inextensible extent of the Earth's land surface which had to satisfy those many demands. While any part of the Earth's surface may have an economic importance through the occurrence of minerals, or for other reasons, the ecumene or habitable surface of the Earth having sufficient heat, moisture and soil to produce crops to support human life, is of limited extent. A fifth of the surface is too cold, a fifth too arid, a fifth too mountainous and a tenth soilless, so that only 30 per cent is potentially cultivable even if the many hazards of soil management in the tropics are overcome: at present each of the world's 2,900,000,000 inhabitants has a share of about 12·5 acres, or 4 acres 'potentially cultivable'. Of this, 1·1 or 1·2 acre is cultivated. Equating non-food crops with the contribution in food from the great open grasslands, it takes the produce of rather more than 1 acre to feed one human being. Against this world yardstick, extreme contrasts can be seen between one country and another—ranging, for example, from Canada with 140 acres a head, 21·5 officially classed as cultivable, with 3·5 used, or the United States (12·5, about 6, 3·0) to England and Wales (0·8, 0·6, 0·55) and Japan, where the food has to be wrested from a cultivated acreage of 0·17 acre a head. In some countries it is clear that concentration on high-calorie foodstuffs and intensive cultivation is a national first priority; in most countries comprehensive land planning is the only sensible approach to the problem. Such planning must be based on objective surveys and mapping of existing land use (hence the importance of the world-wide scheme of the World Land Use Survey of the International Geographical Union) with an attempt to understand the reason for the present position and trends.

Prof. Stamp advocated further attempts to rate land of different types according to potential productivity and compared actual production by using a Standard Nutrition Unit of 1,000,000 calories farm production per annum (equivalent to a food intake of 2,460 per day) pointing out that 'carrying capacity of land' varies in even leading countries from less than 0·3 to 7 such units an acre. He appealed especially for fresh thinking in many directions, and by the many different sciences which have contributions to make. In particular he pointed out the fallacy of discussing 'over-population' unrelated to standards of farming technique—Britain has been over-populated at least since Norman times. A maximum population which could be supported is very different from the small body of experts which might most effectively produce the food. In erosion and flood control more emphasis should be placed on trapping and distributing eroded material where it could provide useful land. While it is desirable to preserve naturally good agricultural land and provide conditions where the microfauna and microflora can do their work without cost, why should not modern earth-moving machinery over large areas mix sterile sands and intractable clays to the right texture to receive the chemist's nutrients?

In the afternoon, after Sir Alexander Fleck and Prof. Kay had spoken, eight or nine speakers who had previously given in their names took part in a general discussion before Prof. P. M. S. Blackett wound up. Ritchie Calder remarked on the historic significance of the occasion—perhaps the first time in 129 years that the Association as a whole had got together to debate a problem of world-wide importance. Science had provided a vast super-market of knowledge: the user must have a thought-out shopping list. The common tacit assumption of the economists that efficiency in farming is to be equated with large-scale monoculture was voiced by Colin Clark, and brought vigorous headshaking from Dr. Kay; further, he doubted whether protein deficiency is widespread and whether many of the policies of the Food and Agriculture Organization are right—especially any encouragement of small-scale intensive mixed farming. Mr. James Callaghan, M.P., sharply reminded the Conference that a 5 per cent increase on prices paid for the agricultural raw materials of the 'underdeveloped' lands more than equalled all 'aid': guarantee their prices and forget 'aid'. Sir Charles Darwin felt that only part of the problem is being considered: man is an animal and behaves as such. With plenty of space there has been unparalleled expansion for two hundred years—the struggle for existence must come. Birth control may stave off the evil day, but is it not possible, he asked, that those who have believed in birth control would be the ones to go under? Prof. Sargant Florence, however, held that we do not want just to keep alive, but at a proper standard: we must increase production, but decrease reproduction.

In his summing-up, Prof. Blackett stressed four points: first, nothing we can do, short of nuclear warfare, can alter the fact that a great increase in population is inevitable in the near future; secondly, the technical 'know-how' exists; thirdly, there must be initiative and 'crash-action; fourthly, the educational aspect is vital and we should open the doors of our universities and technical colleges, however difficult it may be, more widely to overseas students. Finally, he felt that the day's useful discussions had ended on a note of restrained but robust optimism.

L. Dudley Stamp

AFFORESTATION OF INDUSTRIAL WASTE

THE sight of derelict land is all too familiar in the industrial areas of Great Britain, and the purpose of the session which was held in Sub-section K* (Forestry) of the British Association on September 2 in Cardiff was to explore the possibilities of afforestation as a land-use tool to improve the amenities of such areas. The three speakers looked at the problem from different points of view: that of a Forestry Commission officer, a planning officer of a county council, and a land agent to an owner of substantial areas of ironstone workings. It was refreshing to find that there was a large measure of agreement between all three as to where and in what circumstances afforestation could be usefully employed.

The symposium was opened by J. Q. Williamson of the Forestry Commission with a review of the evidence which has been built up over the past fifty years on the planting of trees on colliery spoil heaps. In his opinion the earlier doubts as to the success of such planting because of the probable lack of fertility of such apparently sterile sites has proved groundless. A large range of tree species can be planted successfully on tips so long as certain precautions are observed. The choice of planting species must first be governed by local conditions, particularly by the smoke pollution factor, which may rule out all conifers. Exposure is of great importance in tree growth generally, and the miniature mountains created by many spoil heaps could lead to severe exposure, especially if the heap happened to be already in an exposed position.

In areas where there is atmospheric pollution, birch, alder, sycamore, and in more fertile sheltered areas, ash and poplar, were recommended by Mr. Williamson as the most likely to succeed. Where tips are situated in more rural areas, over which the atmosphere is purer, conifers can also be grown, and this is much more attractive from the economic point of view. Of the conifers, Corsican and Austrian pine are usually to be preferred, but Japanese larch and Scots pine can also do well. His experience has shown that there is no special difficulty in establishing such species by normal forestry methods, but the trees must be looked after. Grazing animals must be rigorously excluded from the young plantations and, most important of all, the local population must be taught to cherish the young trees. Most failures in the past have been due to vandalism, and it is almost a waste of time to embark on a planting scheme unless there is a determined effort to guard the trees from damage during their early years. He recommended strongly that a programme of forestry education for the local population should accompany the planting of any colliery spoil heap, and if possible the local school children should be encouraged to take part in the actual planting and to look on the resulting woods as their own.

Mr. Williamson then gave details of the experimental afforestation of an open-cast coal site in Coed Morgannwg. He pointed out that by applying a restoration specification, which did not entail levelling and restoration of the top soil, a great deal of money can be saved. The result of this work had been most encouraging, and of the species used Corsican pine, Lawson cypress and Japanese larch among the conifers and common alder, grey alder and false acacia among the hardwoods had grown well. The trees on the more porous slopes had grown better than on the level ground where the passage of heavy machines had led to compaction of the soil.

After this technical appraisal of the problem, it was encouraging to learn from Mr. J. Casson, of the Planning Department of the Lancashire County Council, that the extensive work of afforestation carried out by his Department over the past nine years has led him to the same conclusions. He considers that there are thousands of acres in Lancashire alone which could be afforested with comparative ease and at not much, if any, greater expense than that incurred in establishing forest crops on normal sites. It is unfortunate that not many planning authorities in England and Wales have yet made use of Section 89 of the National Parks and Access to the Countryside Act of 1949 to undertake planting work aimed at enhancing the countryside. He considers that while the main objective of such a planting programme is the improvement of amenities, this work could obviously, to a greater or lesser degree, make a contribution to the economy of any area where forestry operations could be successfully carried out. The effect of enhanced amenities in checking emigration and attracting new industry could well be valuable in arresting economic decline; the converse is undoubtedly true—ugliness leads to emigration and discourages new industrial development. He looked on the work done in Lancashire up to the present as being mainly a fact-finding exercise to see how far forestry operations are practicable under various conditions of difficulty (climate, soil, pollution, etc.). They have located their siting in significant localities as experiments, not only in forestry, but also in the use of forestry as an instrument of land-use policy in the overall physical and economic rehabilitation of depressed and damaged regions. His Council's policy has been to acquire or plant under agreement without resort to compulsory purchase. While there is a wide choice of sites which might be planted in Lancashire, it is significant that land acquisition rather than technical difficulties has been the greatest problem in planning a forward programme. For every 100 acres planted, investigations and negotiations have been opened for 200–300 acres which have had to be

abandoned for one reason or another. Shifts in the value of waste materials and land, the presence of 'admitted' claims, conflict in the opencast coal-mining programmes, changes in legislation affecting land values and acquisition procedures, and the psychological factor that as soon as a public authority takes an interest in derelict land its 'value' often rises sharply, have all helped to hinder progress. In spite of this, he thought that it would not be justifiable to use the compulsory powers of the Act in the absence of any overall plan for rural afforestation and with such a wide choice of sites. The same arguments would not be so applicable in urban areas where town plans are well advanced.

More than 300 acres has been planted in Lancashire since 1951 under this scheme, and a great variety of sites has been tackled, varying from colliery spoil heaps, hæmatite waste and Gritstone quarries to degenerate woodland and bare land and moorland. The excellent slides shown by Mr. Casson and his forester assistant, Mr. L. A. King, illustrated very well the progress of the work and, although they had had their failures, it was obvious that, taken as a whole, this well-thought-out planting scheme has been most successful. Fourteen men are now employed on the work and a small nursery has been established to provide the necessary plants. With the development of 'know-how', they are increasingly able to introduce an element of land-scape design into the work and they feel that they are now more successfully harmonizing economic, ecological and amenity aspects within a 'forestry' approach.

In spite of the very encouraging picture that was emerging, Mr. Casson admitted that there is a great deal still to be learned, and he made a strong plea that more scientific research should be done on the biological and technical problems posed by land dereliction, mineral working and the tipping of waste. He thought that such work would be of value not only to programmes of reclamation but also in the greater and increasing field of the planning control of the extractive industries. He realized that it is asking a great deal of local authorities to expect that they should embark on forestry schemes without the technical skills or documented data necessary to bring about a transformation in their derelict areas by using forestry; nevertheless, a great deal more could be done than was being done at present.

It was perhaps appropriate that Mr. Casson's inspiring talk on the tremendous possibilities of extending afforestation on industrial waste should be followed by a very factual account of the successful afforestation of ironstone workings in Northampton-shire on a really large scale. Mr. J. W. Houldsworth, land agent for the owners, Stewarts and Lloyds Minerals, Ltd., quoted chapter and verse of how more than 1,000 acres of land left derelict on 'hill and dale' after the ironstone had been extracted is now carrying flourishing plantations. This woodland area provides considerable amenity value to the nearby industrial town of Corby, as some of the earlier plantations are now 25–30 years of age and looking most attractive. Experience has shown that on the lighter soils a mixture of 40 per cent European larch with 40 per cent sycamore and 20 per cent Scots or Corsican pine is most suitable for the site, and the present intention is to leave the sycamore as a final crop after the gradual removal of the conifers. On the heavier boulder clay, common alder and occasion-ally oak have been planted, but this latter species has been very slow growing.

A permanent staff of 20 men is maintained under a head forester and two foremen. The biggest problem facing the Company is not the establishment of plantations but the extraction of produce from them, especially as the size of pole increases as the plant-ations mature. Up to date there has been no difficulty in disposing of the produce, because a small estate sawmill is maintained for converting it into fencing material for use on the estate and quarries; a large quantity of the second-grade material goes into the steel work for loading strips.

Mr. J. W. Houldsworth was emphatic that, apart from the difficulty of extraction, the establishment and maintenance of plantations on this very unprom-ising waste present no particular problems; in fact there are certain advantages over normal forestry, as no weeding or cleaning is necessary on these bare 'soils'. His conclusion was that afforestation is the most economic solution to the problem of treating some of this worked ironstone land, and that it would prove a good long-term investment.

In the discussion which followed the papers, it was generally agreed that much more use could be made of afforestation in reclaiming industrial waste than had been done in the past. One of the main problems which perhaps makes public authorities a little reluctant to embark on ambitious schemes is the difficulty of making more than intelligent guesses as to what will grow successfully on any particular site. As each site usually varies so greatly within itself, the cost of extensive soil analyses is scarcely justified, and the general opinion of the meeting was that although more research should be undertaken on the constitution and plantability of the various types of industrial waste, there is now sufficient evidence to convince the most doubting authority that at least the easier sites could be planted with a fair degree of confidence.

The most controversial point discussed was the future treatment of plantations on waste land, once they were approaching maturity. Mr. Williamson thought that it would be of no use ever to consider clear felling and replanting, and advocated the introduction of some form of uneven-aged selection system in order that the soil was never bared and the amenity was maintained; but several others felt that there was no reason why such areas should not be clear-felled on maturity and then replanted, so long as attention was paid to shelter belts being left where necessary.

On the extraction problem in the plantations on the ironstone workings, the simple solution of using horses was suggested for at least the small- and medium-sized produce.

The growth of different species was discussed, sycamore and birch in particular, and it was agreed that both these species are difficult to establish; this accords with normal field experience.

This symposium did not minimize the difficulties which are present in the planting of industrial waste, where every site and every part of a site has to be considered separately; but there seems to be no doubt that enough knowledge is now available to encourage public authorities to embark on more ambitious planting schemes than they have ventured on up to date. The inspiring example of Lancashire will, it is hoped, lead to a more vigorous approach being made to this problem in many other parts of Britain.

J. Q. Williamson

INVESTIGATION OF THE UNIVERSE BY RADIO ASTRONOMY*

By Prof. A. C. B. LOVELL, O.B.E., F.R.S.
Jodrell Bank Experimental Station

ALTHOUGH nearly all the major advances in radio astronomy have taken place during the past fifteen years, the basic discoveries were made thirty years ago. At that time Jansky realized that the residual noise in his receiving equipment had a daily sidereal variation and must be the result of radio waves reaching the Earth from outer space, and Appleton in the United Kingdom with Breit and Tuve in the United States, through their studies of the ionosphere, laid the foundation of the radio-echo techniques of radio astronomy. The radio emission from outer space can be received on Earth in the wave-length range from a few millimetres to 10–20 metres. The short-wave end is limited by absorption in the atmosphere and the long-wave end by the ionosphere, and this upper limit tends to vary in sympathy with ionospheric conditions throughout the sunspot cycle. These hindrances will soon be overcome when suitable equipment can be carried in Earth satellites; then it should be possible to determine the true wave-length range of these extra-terrestrial emissions.

Here we are concerned with some of the significant advances made by the application of these techniques to the study of the solar system and the universe, with points of interest from the immediate environment of the Earth to the deepest penetration of the universe.

Some of the earliest successes with the new techniques arose from the study of the transient echoes which are obtained when a radio beam is directed into space. It was shown that these echoes originated from the scattering of the radio waves by the ionized trails of meteoric particles which were evaporating in the height-range of 80–120 km. above the Earth's surface. Methods were developed for the measurement of the velocities of these meteors and later for the determination of the orbits of individual meteor particles. A long-standing controversy was settled when it was shown that the sporadic meteors were members of the solar system and not visitors from interstellar space. New meteor showers active in the day-time sky were discovered and progress has been made in the basic problem of the genesis of this meteoric dust which pervades the solar system.

The extension of these radio echo techniques to the investigation of the Moon and the planets is a more recent activity which required the development of large radio telescopes and powerful transmitters. The lunar work has had two unexpected outcomes which arose from an analysis of the fading of the lunar radio echoes. One has been the discovery that the radio waves are effectively scattered from a rather small portion of the forward hemisphere of the lunar surface; and this led to the demonstration of the possibility of using the Moon as a reflector for round-the-world communication. The other was the proof that a component of the fading arose from Faraday rotation in the ionosphere which has led to an important method for measuring the total electron content along the line of sight from the Earth to the Moon. The attainment of planetary echoes is still a difficult technical problem, and so far only limited success has been achieved in the case of Venus. The future of this work is full of promise since it may involve refinement of the value of the solar parallax, the determination of the rotation period of Venus and preliminary information about the nature of the planetary surface and atmosphere.

The emission from the Moon and many of the planets has now been measured in the radio-frequency range, and this has made possible a comparison of the black-body temperatures in the radio and visual regions. The measurements on Jupiter have been of particular interest for two reasons. First, it appears that the effective size of the disk depends on the wave-length, which indicates that the planet may be surrounded by radiation belts. Secondly, the planet exhibits a strong non-thermal and sporadic type of emission on frequencies below 30 Mc./s. The origin of this emission is unknown although it is believed that it may arise from a relatively few places on the planetary surface.

The Sun has been the most exhaustively studied single object in radio astronomy. Various types of emission in the radio-frequency range have now been delineated. The thermal component originates in the solar corona with an extension which depends on the wave-length. In the centimetric range the effective radius is similar to that of the solar disk with pronounced limb brightening. As the wave-length of observation is increased the effective size of the solar disk increases, until at wave-lengths of several metres the coronal radiation can be detected out to many solar radii. Various types of powerful sporadic emission are associated with sunspots and flares, and the mechanism of emission and the relation to the ejection of particles from the Sun remains an active subject of investigation. The recent results obtained by Simpson of Chicago in the American space probe *Pioneer V* have been of outstanding interest. In this work the cosmic rays from the Galaxy have exhibited a sudden decrease in intensity coincident with solar eruptions, radio emissions and geomagnetic disturbances, when measured at distances of several million kilometres from Earth.

The radio emission from the Milky Way has various origins, the study of which forms a major feature of contemporary research in radio astronomy. The most striking general feature is the resemblance of the intensity isophotes to the concentration of common stars. It has long been realized, however, that the common stars (even if they emit at all) do not contribute significantly to this radiation. The following classes of emission are now recognized:

(*a*) There are a number of localized, discrete sources of emission (radio stars) concentrated near the galactic plane. A few of these have been clearly associated with galactic nebulosities characterized

* Substance of a Royal Society Tercentenary Lecture, delivered on July 23.

by a filamentary gaseous structure with violent internal motion. These include the three known cases of supernovæ (the Crab Nebula, and the supernovæ observed by Kepler and Tycho Brahe), and the most intense radio source in the sky in Cassiopeia. It seems likely that the Cassiopeia source and the other radio nebulosities may also be supernova remnants the initial outburst of which has not been recorded.

(*b*) There is a generally distributed emission observable over a wide range of wave-lengths the isophotes of which are similar to the stellar distribution. At short wave-lengths this emission has thermal characteristics and probably originates in free-free transitions from the movement of electrons in the field of the interstellar protons. At long wave-lengths the intensity and spectrum of the radiation are incompatible with such an origin, and this emission is believed to be synchrotron radiation arising from the relativistic motion of the electrons in the galactic magnetic field.

(*c*) Radio emission is observable from regions far beyond the galactic disk of visible stars. This halo or corona of emission is thought to originate in the same way as the long-wave radiation in the Galaxy, that is, from the relativistic motion of electrons in the galactic magnetic field.

(*d*) In 1951 observers in Leyden, Harvard and Sydney almost simultaneously established the existence of line emission from the neutral hydrogen clouds in the Galaxy on a frequency of 1420 Mc./s. This emission is associated with the transition between two levels in the hyperfine structure of the ground-state of the hydrogen atom. The two levels correspond with the two possible orientations of the electron spin in the magnetic field of the proton. The original suggestion was made in 1945 by van de Hulst, and since the practical demonstration of the existence of this line remarkable progress has been made, particularly by the Leyden school, in observations of the Doppler frequency shift and the derivation of the motion of the galactic hydrogen clouds. This work has given the first detailed insight into the spiral structure of the Galaxy, and is now in process of extension to determine the hydrogen content of extragalactic nebulæ.

An important outcome of the existence of the observable hydrogen line emission is the possibility of measuring the galactic magnetic field by observing the Zeeman effect. The magnetic field should split the line into three components with an extreme frequency difference of 2·8 Mc./s. per gauss. Since the interstellar field is not expected to be more than 10^{-4} gauss the maximum observable shift is likely to be of the order of 30 c.p.s. Experiments are in progress on this problem at Jodrell Bank, and so far an upper limit of 4×10^{-6} gauss has been set for the interstellar magnetic field.

Even a cursory inspection of the intensity plot of a high-resolution radio survey of the sky shows that there are prominent features which are not explicable in terms of the emission from the Milky Way. The most striking feature is the existence of large numbers of localized sources (radio stars) which seem to have a nearly isotropic distribution. A small number of these (two or three dozen) can be associated with the nearer normal types of extragalactic nebulæ. The Andromeda nebula (*M* 31) has been studied in some detail, and as a radio emitter it is found to be essentially similar to the Milky Way, including the existence of an extensive corona around the Galaxy. Studies of the other spiral nebulæ within the range of present-day radio telescopes are still in progress, but the existing evidence is that they also conform to this general pattern.

The problem of the majority of these isotropically distributed sources which are not associated with normal extragalactic nebulæ is believed to be one of the most significant issues in astronomy to-day. It is thought possible that the failure to associate these with visible objects may be because they lie at very great distances in the cosmos. The direct evidence for this arises from a few cases where colliding or interacting nebulæ at great distances have been associated with rather strong radio sources. The most outstanding case is that of the galactic collision in Cygnus at a distance of 700 million light years which is related to the second strongest radio source in the sky. The disparity between the visual and radio luminosity of this source is such that a similar event at ten times the distance could only be observed optically with great difficulty but would still be easily recorded as a radio source. Recently one of these radio sources, the apparent angular diameter of which is about 8 sec. of arc, has been related to a rich cluster of nebulæ at a distance of 6,000 million light years with a recessional velocity amounting to 46 per cent of the velocity of light. The lecture concluded with a reference to the cosmological issues involved in this work, and to the experiments now in progress which are believed to be giving information about the condition of the cosmos at distances of many thousands of millions of light years.

A NEW THEORY OF THE ANGIOSPERM FLOWER

By Dr. R. MELVILLE
Royal Botanic Gardens, Kew

THE interpretation of the Angiosperm flower as a structure built up from modified leaves bearing either ovules or microsporangia dates back to Goethe's essay on the metamorphosis of plants[1]. Most attention and much controversy has been focused on the structure of the ovary, which in its simplest state was supposed to have been produced by the infolding of a leaf bearing ovules on its margin to form a box-like structure similar to the pod of a pea. The carpel theory does account for many of the facts of floral structure; but numerous incongruities are glossed over or avoided by its proponents. Alternative theories have been put forward from time to time, one of the more recent being Saunders's theory of carpel polymorphism[2]. This was shown to be untenable by Eames[3], in a paper in which he expounded the modern form of the carpel theory, that assumes the primitive carpel to have been a palmate leaf

bearing the ovules on its lateral lobes. Even in the comparatively primitive flowers of the Ranunculaceae this theory requires the fusion of vascular tracts in the ovary, which presupposes a long and intricate evolution. Contrary to this, the Angiosperms spring into prominence in the Cretaceous, apparently after a sudden and rapid burst of evolution.

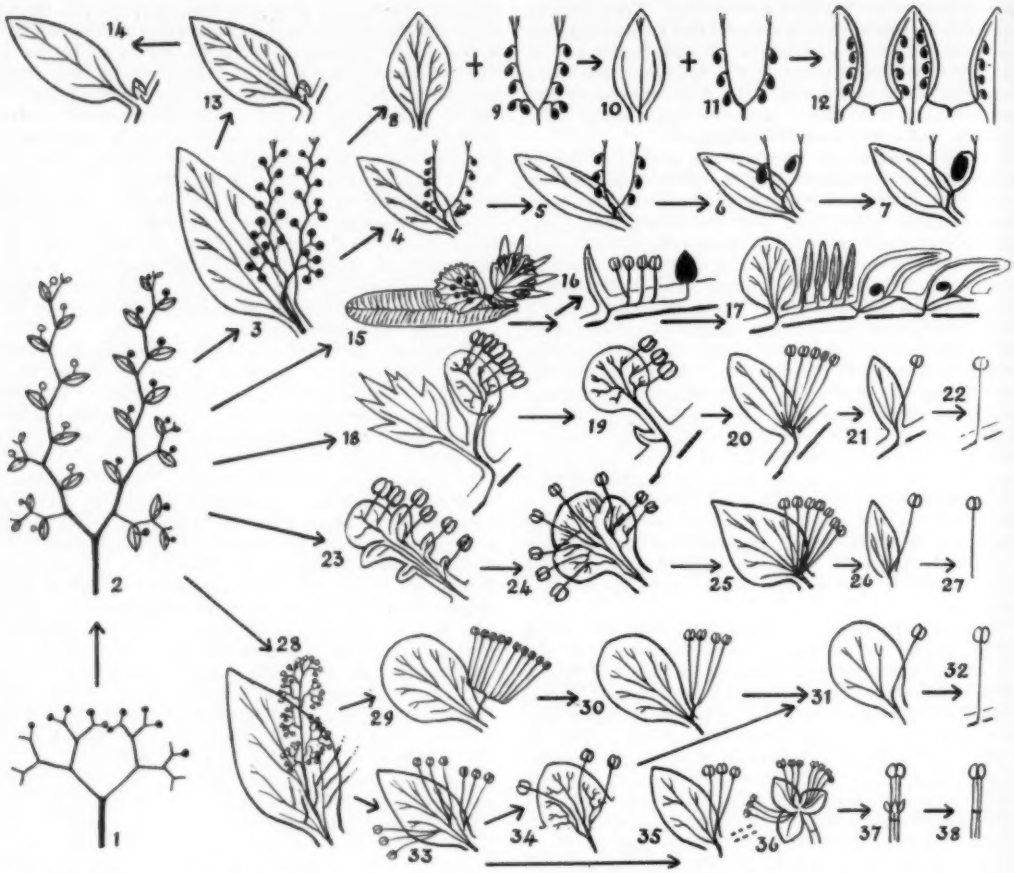
No fossil plants are known with carpels of either the pinnate or the palmate type. Only *Cycas* among recent Cycadales has a megasporophyll with the open pinnate carpel structure; but its microsporophylls bear no relationship to the Angiosperm stamen, and numerous anatomical differences distinguish it and other cycads from the Angiosperms. Although pinnate microsporophylls occurred in flower-like structures in the Bennettitales, the female organs associated with them differed so markedly from the Angiosperm gynæcium that this group, also, cannot be on the Angiosperm line of descent. The failure to find fossil precursors of the Angiosperms is commonly blamed on the paucity of the palæobotanical record. Nevertheless, it is inconceivable that the most successful group of modern plants can have left no trace of its ancestors in the rocks. Darrah[4] believes "that we have most if not all of the clues to Angiosperm origin, but botanists have not been able to put them into a logical story". It is unlikely that they could do so if the carpel theory were based upon false premises.

Starting with the one assumption—that the carpel theory might be wrong—a re-investigation of the vascular structure of the flowers of a range of Angiosperm families has been made. Bleaching and clearing techniques have been used for obtaining preparations in which the complete vascular system could be examined as a three-dimensional object and the exact relationship of its parts determined. Out of this work arose the conviction that the basic structure of the Angiosperm ovary was a leaf bearing on its midrib or petiole a dichotomous fertile branch. From this structure, or its simple derivatives, the gynæcia of all modern flowering plants could be easily formed. The basic structure of the male organs was similar, but, not being restricted by the function of enclosing ovules, more primitive stages in the evolutionary sequence have survived in the andræcium than in the gynæcium. To obviate confusion, it is proposed to avoid the term 'carpel' and to call the basic structure a 'gonophyll'. The male, female and bisexual analogues may then be called 'androphylls, 'gynophylls' and 'androgynophylls'. The theory may be called the 'gonophyll theory'.

For brevity it will be convenient here to present the theory as a 'logical story', leaving the detailed evidence for separate papers on the gynæcium and andræcium to be published elsewhere. The Psilophytales of Silurian to Devonian age may be taken as the starting point. In the simpler members of this group such as *Rhynia* and *Horneophyton*, the plant body was a leafless dichotomous branch system bearing terminal sporangia (Fig. 1). Zimmermann[5] derives all megaphyllous leaves from dichopodia of the Psilophytalean type, by overtopping, planation and fusion. Some of the early stages in these very generalized processes are to be found in the Psilophytales and in the Coenopterid ferns of the Devonian and Carboniferous, while later stages survive in modern ferns and Angiosperms. The development of the megaphyllous leaf used up one of the branchings of each dichotomy, so that, instead of a diffuse branch system, there was a tendency to produce a single main stem bearing a succession of leaves. The main stem dichotomized only occasionally as in *Lyginopteris oldhamia*[6], a habit which has been retained in living cycads and palms. Other plants of the Carboniferous period developed another kind of branching, which proved to be of great significance for the evolution of the higher plants. Early examples occur among Coenopterid ferns, where, as in *Ankyropteris grayi*[6], the petiole trace rounds off to give rise to a stem-like structure shortly after its departure from the stele of the main stem. It then forks, the abaxial part supplying the leaf and the adaxial forming a branch. Thus arose the leaf–branch combination, which formed the repetitive unit in this new kind of branch system.

It is likely that the epiphyllous branch first arose as a reproductive structure, though little fossil evidence on this point has come to light. Excluding adventitious shoots, the only epiphyllous shoots surviving in modern Angiosperms are reproductive in function. An incomplete list of genera in which they are found is given by Velenovsky[7]. The fertile branch may be reduced to a single flower bearing the same relationship to the leaf as the branch of *Ankyropteris*. Examples are to be found in several genera of Epacridaceae, where, as in *Andersonia*, the terminal leaves are fertile and those immediately below have epiphyllous buds in the corresponding position. Here, and in the Chailletiaceae studied by De Candolle[8], the axillary bud appears to have evolved by the sterilization of an epiphyllous inflorescence (Figs. 13, 14). Theoretically, axillary buds could have arisen also by the condensation of compound branch systems composed of the primitive leaf–branch units, but evidence for this alternative is lacking.

The first and sometimes some of the later branchings of the epiphyllous inflorescence in the Chailletiaceae are dichotomous. The main peduncle may be relatively long, or the primary dichotomy may be sessile or sub-sessile on the petiole. The peduncle, also, may vary in position even in a single species, so that at times it may take up a truly axillary position. If the peduncle is then reduced until the dichotomy is sessile, a pair of fertile branches in a single leaf axil results. Both epiphyllous and axillary inflorescences occur in *Dichapetalum pedunculatum* (Aubl.) Baill. and pedunculate and paired axillary inflorescences in *D. timoriense* (DC.) Boerl. and other species. The anatomy of these epiphyllous inflorescences indicates that they are not due to adnation, but that leaf and inflorescence stalk fuse into a single structure, which repeats, in all essentials, the condition in *Ankyropteris*. Further evidence for the view that the Chailletiaceae have retained a primitive form of attachment of the inflorescence is provided by other fossils. Whereas the primitive epiphyllous branch is found in *Ankyropteris grayi* of Lower Carboniferous age, paired axillary branches occur in the later *A. glabra* of Upper Middle Pennsylvanian age[9]. Parallel evolution of simple and paired axillary branches took place in the Cordaitales, where the simple branch is usual, but paired branches occur in *Mesoxylon birame* Baxter[10]. Other groups may have followed a similar path. Rather specialized epiphyllous inflorescences have recently been described by Plumstead[11,12] for the Glossopteridales. There is positive evidence, therefore, for the existence at the end of the Carboniferous or early Permian of plants with epiphyllous, simple axillary and paired axillary



Figs. 1–38. Evolution of the components of the gynæcium and andræcium of the Angiosperm flower: 1, Dichopodium with terminal sporangia, Psilophytalean phase; 2, Condensation to branch system with sporangia epiphyllous on leaflets; 3, Primitive gynophyll, a leaf with complex epiphyllous ovuliferous branch; 4–7, Reduction series giving follicles, legumes and achenes by enfolding; 8–11, Separation of the ovuliferous branch from its blade and reduction; 12, Enclosure of branchlets of ovuliferous branches by alternate whorl of sterile blades or tegophylls; 13, Epiphyllous vegetative bud, Epacridaceous type; 14, Axillary vegetative bud, usual Dicotyledon type; 15, *Glossopteris* (*Scutum*), an androgynophyll with foliaceous male and female branches; 16, *Gnetum*, an androgynous branch adnate to the inflorescence axis; 17, *Magnolia*, androphylls and gynophylls with adnate branches; 18, Catkin scale of *Populus*, an androphyll with cupular fertile branch; 19, *Homalanthus*, cupular fertile branch with reduced bract; 20, *Salix pentandra*, elimination of bract and reduced number of stamens with staminodes; 21–22, Theoretical reduction to single aphyllous stamen; 23–27, Reduction of pinnate androphyll; 23, *Juglans*; 24, *Corylus*; 25, *Carpinus*; 28, Primitive androphyll with many complex branches (one shown), *Ricinus* type; 28–32, Reduction to aphyllous stamen; 29, Tiliaceae (*Sparmannia*); 30, Malvaceae (*Hoheria*); 31, Rhamnaceous type; 32, Ranunculaceous type; 28, 33, 35–38, Euphorbiaceae; 35, *Mercurialis* androphyll; 36, *Mercurialis*, male flower; 37–38, *Euphorbia* spp., male flowers; 34, Rosaceous type androphyll-sepaloid. The examples cited indicate evolutionary stages—not lineages. To avoid confusion, the full complement of stamens is not shown in Figs. 18, 19, 23, 28

inflorescences. The elements necessary for condensation into modern flowers were already present.

In the primitive form of the gonophyll, the leaf–branch unit was repeated many times (Fig. 2). The structure could be condensed in a variety of ways. The male and female organs could be confined to separate parts of the branch system of one gonophyll, or separated completely on different gonophylls. The foliar part of the dual combination could be suppressed in the secondary gonophylls of the fertile part, giving rise to an epiphyllous ovuliferous branch on the primary gonophyll blade (Fig. 3). The same processes, carried further, would produce axillary ovuliferous branches and then ebracteate ovuliferous branches (Fig. 9), which in turn could become associated with sterilized gonophylls in alternating whorls (Fig. 12). Thus could the elements of the modern Angiosperm ovary come into being.

The simplest kind of ovary was formed by folding the blade of the gonophyll around the ovuliferous branch system, which usually dichotomized somewhere near the base. The legumes of the Papilionaceae and the achenes of *Thalictrum* and *Ranunculus* (Ranunculaceae) have this structure, the number of ovules varying from many to one. Fusion of a number of such units gave rise to a multilocular ovary, as in *Oxalis* (Oxalidaceae) and *Triglochin* (Juncaginaceae). In a number of families, the ovary has been formed from sterilized gonophylls, which may be called 'tegophylls', alternating with bifurcated ebracteate ovuliferous branches. With this arrangement, a tegophyll encloses one of the branches of each of the adjacent ovuliferous branch systems (Fig. 12). If the ovuliferous branch bifurcates near the base, the ovary may be a collection of follicles, as in *Caltha* and *Helleborus* (Helleboraceae), or the follicles may be

more or less fused, as in *Butomus* (Butomaceae). When the ovuliferous branch bifurcates at a higher level, the undivided part may take up an axial position and the components of the ovary are clamped together by the enfolding of the adjacent branches in the upper part, as in *Buxus* (Buxaceae). Simple unilocular ovaries with parietal placentation are formed from alternating components by the overlapping of the margins of the tegophylls and the application of the ovuliferous branches to the wall so formed, as in *Berberidopsis* (Flacourtiaceae). Another class of ovary may be formed by simple apposition of the margins of fertile gonophylls. *Koelreuteria* (Sapindaceae) has this structure, false septa between the axial ovuliferous branches and the midribs of the gonophylls helping to hold the components together. A number of modifications of these basic types of structure are to be found in particular families. Similar principles apply to inferior ovaries, with the added complication of adnation and fusion of vascular strands of the ovary with those of the enclosing sepals and petals. This aspect of inferior ovaries is not in dispute and has been described and illustrated by Eames[3].

It is necessary now to return to the primitive androphyll and follow its evolution into modern types of andræcium. Starting with the 'gonophyll of gonophylls' (Fig. 2), a primary gonophyll blade may be retained and the secondary gonophylls with their blades condensed into another expanded leaf-like structure bearing the terminal fertile organs on branches arising directly from its surface and attached to its dichotomous vascular system. This structure is found in the individual male flower of *Populus* (Salicaceae) (Fig. 18), where the vascular system of the 'perianth' branches off from that of the bract, which represents the primary androphyll blade. Essentially the same structure occurs in *Homalanthus* (Euphorbiaceae) where the bract is much reduced (Fig. 19). The bract is eliminated in *Salix* (Salicaceae) (Fig. 20), where the catkin scale represents the secondary blade or the 'perianth' of *Populus*. In *Salix* the number of fertile stamens varies from twelve to two in different species. The nectaries of *S. pentandra* L. are staminodal stumps retaining vestiges of their vascular system, which, by their mode of attachment to that of the bract, indicate their derivation. Further reduction in this series would lead to an androphyll with one stamen (the *Rhamnus* stage—see below) and then to a simple aphyllous stamen (Figs. 21, 22).

If the original compound gonophyll were bisexual, the condensation could take the form of a leaf bearing a pair of leaf-like structures, one male, the other female. These would be opposed on the primary dichotomy of the epiphyllous branch and their ovules and micro-sporangia would be attached to the opposing surfaces. This structure is exactly that of the *Glossopteris* fructifications (Fig. 15) of Lower Permian age recently described by Plumstead[11,12]. Although palæobotanists have not unreservedly accepted her interpretation, the continued existence of similar unisexual structures is in its favour. Moreover, the presence of a once bifurcated branch arising in the opposed position on the adaxial side of the vascular system of the 'perianth' in the staminate flower of *Populus canescens* L. (Fig. 18) is in the position it would need to be if it were derived from a sterilized female branch. Thus the possible origin of the male flower of *Populus* from a primitive bisexual structure of the *Glossopteris* type is an open question. The present evidence suggests that *Glossopteris* must have been very close to the pro-angiosperm stock at that level of evolution.

The bisexual condition is found also in the androgynous inflorescences which often occur on male plants of *Gnetum*[13]. They probably represent the primitive condition in the Gnetales. Here, there is a fertile branch attached to the vascular trace of the bract, but it is adnate to the inflorescence axis (Fig. 16). Allowing for the modifications due to adnation, this arrangement is similar in essentials to that found in the Glossopteridales and suggests a relationship with that group. Adnation of the fertile branch to the main axis is found again in the flower of *Magnolia* (Fig. 17). The sepal of *Magnolia* is an androphyll bearing stamens in a manner similar to *Gnetum*, but the tip of the fertile branch trace continues upwards and fuses with a small branch arising from the ovuliferous branch of the follicle above. The latter is produced by the enfolding of a gonophyll of the type shown in Fig. 6. After the anastomosis, the branch trace continues upwards and fuses with the corresponding branch of the follicle next above. In this way, the main stele of the floral axis is enclosed in a network of vascular traces resembling a dictyostele, through the meshes of which the follicles protrude. The similarity of the staminate part of the *Magnolia* flower to that of male *Gnetum* in the essentials of its vascular structure suggests that the Gnetales and the Magnoliales are divergent offshoots of a common stock. Thus, there is at last evidence to link the Dicotyledons with the Gymnosperms through their reproductive organs.

Another type of condensation of the primitive system could lead, by overtopping and planation, to a pinnate leaf-like structure bearing fertile branchlets on the leaflets. Reduction of the fertile branchlets to single stamens would then lead to the condition found in the individual catkin scales of *Juglans* (Juglandaceae) (Fig. 23). Further reduction of this to the three-lobed condition would give the catkin scale of *Corylus* (Corylaceae), in which two stamens are borne, one each on the two main branches of the vascular system of the terminal lobe and three on each of the lateral lobes (Fig. 24). There is no aggregation here into a flower-like structure, but *Alnus* (Betulaceae) is more advanced in this respect. In it, the subtending scale bears three flower-like structures consisting of four scales, each with a single epiphyllous stamen. Further condensation in the Corylaceae leads to the fusion of the three lobes of the catkin scale into one in *Carpinus* (Fig. 25) with relegation of the stamens to the basal portion of the vascular system. The logical end-points of this sequence, again, would be an androphyll with a single stamen and then an aphyllous stamen (Figs. 26, 27).

Yet another type of condensation of the original compound gonophyll would lead to a flat blade with several fertile branches of one sex attached to it. Such is the condition of the perianth lobes of the male flowers of *Ricinus* (Euphorbiaceae) (Fig. 28). The complex staminal branches are dichotomous systems in which the terminal branchlets bear a pair of anther sacs on either side of a minute leafy blade, here interpreted as the ultimate androphyll with a pair of microsporangia. Numerous stages in the reduction of this complex androphyll occur in the Euphorbiaceae, leading to androphylls (perianth lobes) with three or four stamens in *Mercurialis*

(Figs. 35, 36) and three, two or one in *Dalechampia*. These male flowers have an absciss layer below the perianth. They are borne in complex inflorescences capable of a second cycle of reduction, which may lead from a flower of the *Mercurialis* type to one with three perianth lobes and one stamen (Fig. 37) and thence to the unistaminate male flower of *Euphorbia* (Fig. 38) with no perianth, but an abscis layer in the middle of its stalk[14].

Reduction of an androphyll of the *Ricinus* type could take two other paths. In one, the compound branches would be reduced to single stamens, so producing a blade with a variable number of simple stamens distributed over its surface. A whorl of five of these relatively primitive structures fuse together to form the sepals and perigynous cup of the Rosaceae (Fig. 34). The petals in this family are sterile structures, probably evolved from androphylls of the same kind. The second type of reduction leads to an androphyll bearing in a median position on its surface a single dichotomous branch system, each branchlet terminated by a stamen. This condition occurs in the Tiliaceae (Fig. 29) and Malvaceae (Fig. 30), where the stamens are grouped in dichotomous systems attached to the petals, with filaments free in the one and adnate with those of adjacent petals into a column in the other. Either of these types of androphyll could lead by further reduction to a single epipetalous stamen, as in *Rhamnus* (Rhamnaceae) (Fig. 31) and finally to a single aphyllous stamen attached at a 'leaf-gap' to the floral axis, as in *Ranunculus* (Fig. 32).

The vascular structure of the flower provides evidence for a number of evolutionary lines within the Angiosperms. The primary diversification of the pre-angiosperm stock appears to have taken place during its gymnospermous phase in the late Carboniferous or early Permian. Many Angiosperm lineages must date back to this period as distinct lines of evolution, so that, in this sense, the Angiosperms are polyphyletic. Already the new theory is throwing much light on Angiosperm phylogeny. The great disparity between the floral vascular systems of Ranunculaceae and Magnoliaceae, indicating an origin from widely divergent modifications of the gonophyll structure, implies a very ancient separation of these stocks. A number of other lineages must be as old.

From its mode of origin it might be anticipated that derivatives of the gonophyll would be found in parts of the plant other than the gynæcium and andræcium. Reference has been made above to staminodal nectaries, but the nectarial flap of the *Ranunculus* petal is not obviously of this origin. It is often provided with a vascular trace[15], which is opposed to the petal trace, as it would be if it had originated from an epiphyllous branch. The petal of *R. gunnianus* Hook. has several nectarial flaps, which indicates the probable origin of the *Ranunculus* petal from an androphyll of the rosaceous type (Fig. 34). Other structures with opposed vascular systems are the coronas of the perianth of *Narcissus*[16] and of the sepals and petals of *Passiflora*[17]. In the latter, the vascular system of the corona is often elaborately dichotomous and apparently little altered from that of its gonophyll precursor.

The flower and inflorescence are both condensation products of the primitive gonophyll of gonophylls. As in the flower, condensation of the inflorescence has taken many paths. Sometimes evidence of the epiphyllous origin of the fertile branch is retained in the attachment of the flower to some part of the vascular system of its bract (Epacridaceae, Proteaceae). The compound female inflorescence of *Dobinea delavayi* Baill. (Anacardiaceae)[18] provides a striking example in which the individual flowers are attached to the centre of a rounded dentate bract reminiscent of the fertile scale of *Glossopteris* (*Scutum*). Allusion has been made already to the occurrence of epiphyllous inflorescences in a number of families. DeCandolle's study[6] of the Chailletiaceae indicated that the epiphyllous inflorescence of this family is not adnate to the leaf, but is part of it and that all stages in the detachment of the inflorescence from its leaf occur, leading to the axile position. Similar evidence is to be found in other families.

In this brief summary of the gonophyll theory it has been possible to cite comparatively few examples. It is hoped, however, that enough has been said to illustrate the scope of the theory in explaining not only the gynæcium but also other parts of the flower and the inflorescence. Many facts that formerly appeared to be anomalous in the context of the carpel theory now fall naturally into place. For the first time it is possible to indicate a link between the reproductive structures of *Gnetum* and those of Angiosperms and to point out similarities between those of *Glossopteris* and the andræcium of certain Angiosperms.

In conclusion, it is my pleasure to acknowledge the help of my daughter, Mrs. F. A. Wrigley, in making preparations for the study of the gynæcium, and of my colleagues at the Royal Botanic Gardens, Kew, for many stimulating discussions and for directing my attention to relevant plants and literature.

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OBITUARIES

Dr. C. E. Kenneth Mees, F.R.S.

With the death of Dr. C. E. Kenneth Mees on August 16, at Honolulu, Hawaii, at the age of seventy-eight, the world has lost a major pioneer of industrial research. To-day industrial research is fashionable, and many good scientists are attracted to it. Things were very different in 1906 when Dr. Mees joined the small firm of Wratten and Wainwright, makers of photographic plates. He had just emerged from a brilliant university career with degrees of B.Sc. and D.Sc., both by thesis in collaboration with Samuel E. Sheppard under Sir William Ramsay of University College, London. Sheppard adopted the

more usual course and went to Germany to continue academic research, though he rejoined his old colleague later to continue an association which lasted throughout Sheppard's life. Mees turned to industry for reasons which he afterwards stated to be political. The son of a Wesleyan minister, he was a Fabian who realized that an equal sharing of wealth, while effectively depriving the rich, would do little to benefit the poor. What was needed was a general increase in the standard of living, and this he believed could be achieved only by the application of science to industry. An experience from the Wratten and Wainwright days can now be seen to epitomize what he considered should be the objectives of industrial research. Mr. Wratten had told him that plate prices were being cut, profits were diminishing and that he must devote his energies to reducing their manufacturing cost. Mees completely ignored these instructions. The blue-sensitive plates of the day were being bathed by the user in solutions of dyes to make them sensitive to longer wave-lengths. He found out how to incorporate this step during manufacture and proceeded to market outstandingly good panchromatic plates at a premium price with great benefit to the Company's profits. Throughout his subsequent career he was always aiming to open up new markets with new products; others could worry about holding existing markets. In another way these early years in industry set the pattern of what was to come, in that despite his many preoccupations with commercial problems, he found time to publish several scientific papers of importance.

He had made himself so indispensable to Wratten and Wainwright's that when, in 1912, George Eastman wanted his services in order to found a research laboratory, he had to purchase the Company. His subsequent career with the Eastman Kodak Co. continued until he retired in 1955, seeing him appointed a director in 1923 and vice-president in charge of research in 1934. He became internationally recognized as a pioneer in industrial research and a great exponent of it. The reputation of his laboratory in Rochester, New York, grew by his insistence on high standards and on the publication of the results of research. The decision whether to publish or not was based on the principle that results of value to science ought to be published. This was in line with his belief that industry should repay its debt to pure science, in accordance with which belief he undertook to supply special—and unprofitable—plates to astronomers and started the manufacture of Eastman organic chemicals when the United States was cut off from German supplies during the First World War—a service still valued by organic chemists everywhere. He established research laboratories in the Company's subsidiaries in England and in France and was a frequent visitor to Europe.

Mees's influence on the commercial success of the Company was equally great, and he was largely responsible for such outstanding contributions as the introduction of amateur cinematography and for the popularization of colour photography in all its forms. Of his personal work, apart from many scientific papers, must be mentioned one of the earliest books on the organization of industrial research, published in 1920, which was brought up to date much later by the publication in collaboration with John A. Leermakers of "The Organization of Industrial Scientific Research". His greatest contribution to scientific literature was the "Theory of the Photographic Process" (1954), which had its origins in the book published in collaboration with Sheppard based on their research theses in 1907 which became known simply as "Sheppard and Mees". Recognition of his work was made plain by his receiving from almost every scientific body connected with photography their highest awards, by his election to the fellowship of the Royal Society (1939) and to membership of the U.S. National Academy of Sciences on becoming an American citizen in 1950.

Perhaps Mees's outstanding characteristic was his insistence on the importance of facts and his ability to analyse their implications. It was for this reason that in debate he could be a devastating opponent, but if it could be shown that the facts were against him he cheerfully accepted, not defeat, but the new circumstances. Mees usually dominated any group in which he found himself, mainly by the clarity with which he saw any problem (and the solution to it) and the consequent forcefulness with which he could express himself. He once gave the advice to one of his men, who was writing a book: "If you don't know for certain, don't say it; if you must say it, be dogmatic!" He was widely read, an authority on many subjects, ranging from astronomy to the history of ancient Egypt, having as a central theme the growth of science and technology through the ages. This interest led to the publication in 1946 of "The Path of Science", written in conjunction with John R. Baker. He was a brilliant lecturer, as those who remember his Royal Institution Christmas lectures in 1935 will know, and a stimulating conversationalist. His views on the organization of industrial research were widely sought by other directors of research and by industrialists. They can well be summed up as insistence on the importance of the individual. He had no use for the idea that research had to become a team effort, and he believed that the youngest of men could do worth-while research if given the opportunity, as he had indeed proved in his own case. He surrounded himself by many brilliant individualists, gave them the freedom such men require, and regarded his job as the protection of these men from the direction of others rather than the direction of their work by himself. He asked the Company to judge by the end-results. It had no regrets.

Mees married in 1909 Alice Crisp (she died in 1954), by whom he had a son and a daughter.

E. R. DAVIES

Dr. R. C. Farmer, O.B.E.

THE death of Dr. R. C. Farmer on July 30 at the age of eighty-two has removed one of the great pioneers from the world of military explosives. He was well known not only for his contributions to the defence of the State but also for his publications, which appeared over a number of years in scientific journals. It is remarkable that his last three papers were printed in the *Journal of the Chemical Society* when he was within a few weeks of eighty-two. He married, in 1908, Maud Mary, daughter of Mr. T. T. Sharpe, of Liverpool, and had two sons and one daughter, all of whom have taken up scientific work.

Robert Crosbie Farmer was born on November 30, 1877, in Liverpool, where he received his early education and later graduated with first-class honours at the University. With the aid of scholarships he studied at Würzburg under Hantzsch and Röntgen, and was awarded the Ph.D. (*summa cum laude*) for

his work on the tautomerism of hydrazones and oximes. After a short spell as analyst in a paint works, he entered the University of Birmingham with a Priestley scholarship and worked on the use of liquid nitric peroxide as a solvent for organic compounds. He also developed a new way for measuring hydrolytic dissociation (partition method). Afterwards he was appointed lecturer and demonstrator and was awarded the degree of M.Sc. (Birmingham and Victoria).

In 1902 he became principal assistant to the late Dr. O. J. Silberrad at the Experimental Establishment of the War Office Explosives Committee (later Research Department, Woolwich), of which Lord Rayleigh was president and Sir William Crookes a member. His earliest work was on the mechanism of the decomposition of nitrocellulose and on cordite Mk. 1, which had ignited spontaneously in several magazines in India. This work was embodied in his thesis for a D.Sc. (Liverpool). When Dr. Silberrad left in 1906, the vacant post was offered to Dr. Farmer, but serious ill-health at that time prevented his acceptance and Dr. (later Sir) Robert Robertson was appointed. During the ensuing years he continued his work on the chemical stability of explosives and developed the vacuum stability test for high explosives, a test which is now regarded of first importance in considering their suitability for Service use. When War started in 1914, he devised a process for the manufacture of trinitrotoluene which was used on the largest scale in Government factories and by several private firms. Early in 1915 he was transferred to the Department of Explosives Supply and became chief chemical adviser to the director general, Lord Moulton. In this capacity he was responsible for guidance to manufacturers and safety of processes, several of which he improved. He had control of extra-mural research in this field at the universities and was a member of many committees, including those on Munitions Inventions and Nitrogen Products.

At the conclusion of the War in 1918 he was made O.B.E. and received a Russian decoration, the Order of St. Anne. He left the Government service, forfeiting all rights to pension, and became a director of W. J. Bush and Co., manufacturers of fine chemicals. In 1922 he accepted an invitation to return to the Research Department as deputy director of explosives research and later became chemical adviser. In these capacities he was largely responsible for organizing researches and dealing with a multitude of technical problems arising in the Fighting Services. He represented the Department on a number of committees, including those on Adhesives and Forest Products under the Department of Scientific and Industrial Research, and for some years was a member of the Council of the Institute of Chemistry. On retirement he continued research work at King's College, London, up to his last years.

Dr. Farmer was of a lovable disposition and sacrificed all considerations of self to duty. He had abundant wit and his speeches on lighter occasions were masterpieces. His recreation was walking. No one can be more indebted to him for his generously given help through strenuous years than myself.

Godfrey Rotter

NEWS and VIEWS

Bacteriology at University College Hospital Medical School: Prof. Wilson Smith, F.R.S.

Prof. Wilson Smith is retiring from the chair of bacteriology at University College Hospital Medical School, a position he has occupied since 1946. Prof. Smith is a Manchester graduate, having qualified in 1923. In 1927 he went to work at the National Institute for Medical Research at Hampstead and soon came to the fore as a contributor to knowledge about viruses, particularly vaccinia and herpes. In 1933, with P. P. Laidlaw and C. H. Andrewes, he demonstrated that influenza virus would infect ferrets, a discovery which opened the way to a laboratory investigation of the disease; and in the following years these three workers laid the foundations of present-day knowledge of influenza in its laboratory aspects; they soon found that the virus would infect mice, could be grown in fertile eggs and in tissue culture and could be studied by means of various serological tests. In 1939 Wilson Smith was appointed to the chair of bacteriology at the University of Sheffield, but returned to London in 1946 to the chair which he is now vacating. With various collaborators he has continued to work on influenza, particularly its serological aspects, and on poliomyelitis. His contributions have not only been to virology, however, but also to bacteriology. He was elected to the fellowship of the Royal Society in 1949 and has twice served on its council. He delivered the Society's Leeuwenhoek Lecture in 1957 on "Virus-host Cell Relationships".

Messrs. Macmillan announce that, as from January 1, 1961, the subscription price for *Nature* will be £8. 8s. per annum and *pro rata*. The price of single issues remains 2s. 6d.

Prof. G. Belyavin

Prof. Wilson Smith is succeeded in the chair of bacteriology in University College Hospital Medical School by Dr. George Belyavin, who is at present senior lecturer in that department. Dr. Belyavin was educated at St. Paul's School and the Westminster Hospital, from which he graduated M.B., B.S. in 1943. He was awarded the M.D. in 1960. During 1944–46 he acted as resident pathologist to the Westminster Hospital, and from 1946 until 1949 he was a member of the Department of Biological Standards of the National Institute for Medical Research, Hampstead. Since 1949 he has been continuously at University College Hospital Medical School. Dr. Belyavin has had two main research interests—immunology and virology which, as his

published work shows, he has been able to combine successfully. He has been concerned with the mechanism of the antibody response, and in collaboration with Prof. Wilson Smith he has studied the kinetics of the formation of different types of antibody in response to infection with poliomyelitis and influenza viruses. Recently, he has investigated the immunological response in Hashimoto's disease in collaboration with Dr. W. R. Trotter, and has identified the antigen responsible for the positive complement fixation test in this disease. A major interest for some years has been the study of the antigenic characters of influenza viruses and factors in normal sera which combine with these viruses.

Atomic Energy Research Establishment: Solid State Physics Division

A SOLID State Physics Division has been formed at the Atomic Energy Research Establishment, Harwell. Its initial term of reference will be: to carry out basic research leading to greater knowledge and understanding of the structure and behaviour of solids. Dr. W. M. Lomer, at present head of the Theoretical Physics Division, has been appointed head of the new Division. Dr. W. C. Marshall, at present a group leader in the Theoretical Physics Division, is to succeed Dr. Lomer as head of the Division.

The formation of the new Solid State Physics Division reflects the importance which the Authority attaches to a better understanding of the structure and properties of solids. The nuclear reactors at Harwell, and particularly the research reactor *Bepo*, and the materials testing reactors *Dido*, *Pluto*, play a very important part in studies of the properties of materials. The programmes of work fall broadly into two parts. First, there are basic and applied studies of the effects of nuclear radiation on materials of decisive importance to reactor technology: this work will remain under the control of existing Harwell Divisions, particularly the Metallurgy Division. Secondly, there are more basic studies of the behaviour of solids in general, without reference to any particular application in reactor technology: this work will be the special concern of the new Division. It will include studies of the formation and nature of defects in crystals; other physical properties of metals, alloys and magnetic materials; and fundamental crystal physics using extracted beams of neutrons from the Harwell reactors.

The International Atomic Energy Agency

IN a statement issued in Vienna on the third anniversary of the establishment of the International Atomic Energy Agency, Mr. Sterling Cole, its director-general, said that the experience of three years had demonstrated that a balanced development of the peaceful uses of nuclear energy could be achieved only by international co-operation on the widest possible basis. It was now apparent that the development of reactors, and in particular power reactors, would take considerably longer than was originally expected, but progress in the use of radioisotopes and other radiation sources had been rapid and substantial, especially in industry and medicine. Early hopes that nuclear power would soon become an effective instrument for the rapid industrialization of under-developed countries had been somewhat hampered, and in assisting such States the Agency at this stage was more concerned with preparing the groundwork than with advanced projects. Technical assistance and training were now two of its most important programmes; the exchange and dissemination of information was another developing function; and its research problem was mainly concerned with problems of radiation protection.

The Russian Earth Satellite 1960λ

REFERENCE was made in *Nature* of September 3, p. 830, to the Earth satellite (1960λ) or *Sputnik* 5, on the basis of reports then available. This satellite was launched on August 19 at about 0845 hr. U.T., and consisted of the 'space capsule' itself, and the last stage of the launching rocket. The two objects were seen from the United Kingdom at about 2113 hr. U.T. on August 19 with apparent magnitudes of + 3 and + 6, the fainter object following the brighter in the same orbit, with a separation of about ½′— whether the brighter or the fainter object was the 'capsule' is not certain. Transmissions from the satellite on frequencies of 19·995, 39·990 and 59·985 Mc./s. were received by staff of the Radio Research Station, Slough, during transits occurring at about 1322 hr., 1510 hr., 1938 hr. and 2113 hr. U.T. on August 19. Signals from the satellite were also received at the Radio Research Substation, Singapore, at about 0800 hr. U.T. on August 20, but were not received at Slough later that day when the satellite was next due to be in the neighbourhood of Great Britain. This was explained by a *Tass* report of August 21, in which it was reported that the space capsule was brought out of orbit from a height of about 300 km. soon after 1200 hr. U.T. on August 20, having performed 18 revolutions.

Effects of Autonomy on Science and Technology

UNDER the somewhat misleading title "Local Self-Government", Political and Economic Planning has issued an admirable objective analysis of the experience of such regions of the United Kingdom as Wales, Scotland and Northern Ireland, and also the Isle of Man and of the Channel Islands (Vol. 26, No. 444 (12th September, 1960). Pp. 229–280. (London: Political and Economic Planning, 1960.) 5*s*.). That such an impartial account of the varying degrees of independence and interdependence of the governments of these territories and assessment of the present position scarcely touches on scientific matters as such in itself points to the spurious character of some of the claims for separate national scientific institutions or departments, and although the conclusions drawn touch only on the political and administrative aspects of autonomy, the broadsheet provides a reasoned and impartial appraisal of the arguments for and against autonomy, and of the difficulties and advantages that may well be of service to the scientist or technologist confronted with such claims. It is a real contribution both to the understanding of the present situation and to clear thinking about the real issues involved. The arrangements described are complicated, but it is clear that they are understood by interested and informed opinion in the territories concerned and that while the measure of autonomy enjoyed is varied, it is what it purports to be.

Survey of Information/Library Units in Industrial and Commercial Organizations

THE results of a survey of the information/library units of some 52 British industrial and commercial

organizations made in 1956–58 by Dr. D. J. Campbell are analysed in a report by Mr. C. W. Hanson, now published by the Association of Special Libraries and Information Bureaux (Pp. vi+42+vi. London: Aslib, 1960. 12*s*. 6*d*.; Aslib members 10*s*.). The survey was undertaken to provide factual data on the size, scope, organization, methods and cost of such units in the United Kingdom and it is based on a 12 per cent sample. Two-thirds of the units surveyed were responsible to a head of research or to a director, the remainder mostly to a senior technical officer. In terms of employees or staff the smaller organizations had relatively larger units than the larger organizations; the number of people served per information/library worker averaged 54, ranging from less than 20 to 200. 58 per cent of the heads of units were either graduates or chartered librarians or both. There were more men than women heads, but more of the women were qualified, and among the supporting staff there were three women to one man. Salaries and wages of staff accounted for about three times as much as the purchase of documents. Accommodation averaged 200 sq. ft. per member of information/library staff, but two-thirds of the organizations outside of London exceeded this figure. Two-thirds of the organizations took between 100 and 300 periodicals, the median number being 210, and one in twelve was in a foreign language; 16 per cent were later bound and 25 per cent were kept indefinitely unbound. Data on books were insufficient to permit any deductions, and only 6 out of the 52 units were responsible for the present organization's technical correspondence files. About half the units scanned national or local newspapers; 37 per cent possessed a microfilm reader and 8 per cent a microcard reader. About half used the Universal Decimal Classification, the rest using their own schemes. The main catalogue of 35 units comprised a classified index on cards; only one used a punched card subject index. Documents borrowed from outside libraries for 27 of the units averaged 12 per cent of the number lent from their own stock. About half did little or no stocktaking, and 48 units employed 23 different methods for recording loans. Only one-third could report how many inquiries they handled, but 90 per cent issued some kind of serial publication, commonly lists of new books and selected literature references, with abstracts.

Kirkia: a New Herbarium Journal

The aim of *Kirkia*, the new journal of the Federal Herbarium, Salisbury, Rhodesia and Nyasaland (editor, H. Wild. From the Government Printer, P.O. Box 8062, Causeway, Salisbury. £1 10*s*. per annum), is to provide botanists in these regions, and elsewhere, with a readily available source of taxonomic information on a floristic region of very considerable interest; and to afford a medium for relevant botanical publications. To quote: "With regard to the aims of the journal our intention is to confine ourselves to articles concerning African botany and to give priority to articles dealing with taxonomy, floristic studies, phytogeography and the history of botany and botanical exploration which have some bearing on the flora of the Federation. Ecological articles may be published but usually only if they are of a general nature. As space permits we will welcome articles on these subjects relating to other African territories and particularly those territories which are our near neighbours". The name chosen for the new journal commemorates the work of Sir John Kirk (1832–1922), who, as botanist, accompanied Livingstone to the Zambesi, Victoria Falls, Lake Nyasa, etc.

Museum of Comparative Zoology, Harvard

The annual report for 1957–58 of the Director of the Museum of Comparative Zoology at Harvard College records progress in its usual activities of research, instruction to graduate students and curatorial routine (Pp. 28. Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1960). The year was also marked by rather unusual activities such as expeditions by members of the staff to Asia, Australia and South America. Especially noteworthy of mention was a substantial grant from the National Science Foundation for the improvement of the collections and other facilities. The grant is to be spent over a five-year period and much of it will be used on scientific and technical assistance to bring the collections into good shape. Microscopes, other types of equipment and badly needed storage cases were also purchased. The report concludes with an impressive list of publications.

The National Museums of Southern Rhodesia

The chief item of interest in the annual report for 1959 of the three museums which comprise the National Museums of Southern Rhodesia is an account of the handing over of the Umtali Museum. This was previously owned by the Umtali Museum Society, but is now administered by the Trustees of the National Museums of Southern Rhodesia. The Municipality of Umtali has allocated a one-acre site to house the material collected by the Society and also made a generous grant. The staff has been reorganized so that the Director of the Museums is now relieved of detailed administration of any one Museum and can devote his energies to overall planning.

New Electronic Teaching Apparatus

The Griffin-ClarkeSmith electronic teaching apparatus 'Mechtronics' is a set of apparatus designed for easy assembly by pupils. A minimum amount of manual and technical skill is demanded of the user, since soldered joints are eliminated and the assemblies are screwed or clipped together on a peg-board layout. The complete set contains radio valves, transistors, a photoelectric device, and other electrical components. For qualitative experiments batteries only are required. For quantitative experiments external instruments are required together with suitable power supplies. The 'Mechtronics' set covers the requirements of the revised syllabus for the General Certificate of Education at Ordinary and Advanced Levels, and may be used by the enthusiast for a wide range of other electronics experiments. The apparatus is obtainable from Griffin and George, Ltd., Ealing Road, Alperton, Middlesex.

American Palms Illustrated

The Chicago Field Museum of Natural History, which has issued many admirable volumes on the flora of Central and South America, including B. E. Dahlgren's "Index of American Palms" (14, Bot. Ser., pp. 438; 1936), has now produced a companion volume of palm illustrations (Dahlgren, B. E., 14, 412 plates; 1959. 10 dollars). These consist of

photographs of type specimens and of hitherto unpublished plates of Liebmann's elegant drawings, each with its appropriate references and annotations. This handsome volume should be of great value to workers in systematic botany remote from herbarium resources.

Geochemical Landscape

A 'GEOCHEMICAL landscape' is a term introduced by B. B. Polynov for a geochemical characterization of a given part of the Earth, as based on a joint influence of climate, relief, geological structure and vegetation, on the chemical processes occurring in this region and especially on the prevalent chemical elements and of their migration and accumulation. A. I. Perelman (*Priroda*, **3**, 23; 1960) emphasizes that the main geochemical features of a geochemical landscape are determined by the biological cycle, although there are geochemical landscapes, like those of the polar or desert type, which are more or less abiogenic. For a brief characterization of a geochemical landscape, Perelman proposes a special geochemical formula, giving first the typomorphic elements and then, above the bar the element deficient and, below the bar the elements in excess of the average. The author emphasizes the importance of the study of geochemical landscapes for agriculture, the study of ore deposits and population health. A map in colour to the scale 250 km. = 1 cm. of the geochemical landscapes of the Soviet Union is given.

Medicinal Plants of the Arid Zones

UNDER this title a new investigation has recently been published under the auspices of Unesco ("Arid Zone Research." Pp. 13. H.M. Stationery Office, June 1960. 15*s*.). It will be noted that some twelve volumes in the Unesco Arid Zone Research Series have already been issued. These include digests of research on major topics such as plant ecology, hydrology, etc., and also monographs on particular subjects. In the present publication, which is of interest both to botanists and pharmacologists, indications are given of the directions in which research on medicinal plants is tending and of the economic possibilities which the utilization of such plants may afford. Dr. I. C. Chopra, of the Drug Research Laboratory, Jammu, India, has been primarily responsible for the botanical aspects, and Prof. R. Paris, of the Faculté de Pharmacie, Paris, for pharmacological aspects. The monograph contains a list of medicinal plants found in the arid zones. In the botanical section, following some general introductory matter, the genera and species of interest in pharmacy are set out alphabetically, briefly described, and their special properties and the relevant literature indicated. In the pharmacological section, the therapeutic or other commercial properties of the species are indicated, together with notes on local usage, artificial synthesis and the scope for further work on, and utilization of, the natural product. Some species possessing medicinal properties, on which no scientific work has yet been done, are mentioned. This section not only contains many items of considerable interest, but also affords a valuable brief survey of an extensive field of traditional and contemporary knowledge.

Eichhornia crassipes

H. R. J. DAVIES (*Nature*, **184**, 1085; 1959) and N. W. Pirie (*Nature*, **185**, 116; 1960) suggest that the danger of the presence of *Eichhornia crassipes* in the Nile is over-rated, that uses might be found for the plant, and that research should therefore be concentrated in that direction rather than merely on the problem of its eradication.

A meeting was organized by the Scientific Council for Africa South of the Sahara in Leopoldville in 1957 to consider problems related to the spreading of this weed. This meeting was attended by specialists from Belgium, France and the United Kingdom, and was presided over by Prof. J. Lebrun, secretary-general of the Institut National d'Etudes Agronomiques au Congo and vice-president of the Scientific Council for Africa South of the Sahara, who is well known for his work in the field of tropical botany. The agenda of this meeting covered the following topics: biology and ecology of *Eichhornia crassipes* in tropical Africa; its effect on natural plant communities and other organisms; results of direct control measures; means applied to achieve control; effects of treatment on natural vegetation and on riverine crops; treatment in relation to climatic or hydrological conditions; utilization of *Eichhornia crassipes*; measures to be taken to avoid invasion by other aquatic pests. The recommendations made by this meeting (*Eichhornia crassipes*, CCTA/CSA Publication No. 27; PMB 2359, Lagos, B.P. 949, Bukavu) have served as the basis of the action undertaken by the Member Governments of the Commission for Technical Co-operation in Africa South of the Sahara in this field. That referring to the possible utilization of *Eichhornia crassipes* states: "The Symposium, bearing in mind that considerable research work and a number of experiments have been carried out in all countries affected by this pest, considers that there is no need to pursue such experiments. It draws attention to the danger of the dissemination of seeds by cattle food and composts produced from *Eichhornia*".

Leaf-Shape in Hybrids

IN a metrical study of leaf-shape in some F_1 hybrids and their parents, R. Melville has examined the proposition that the leaves of hybrids tend to be intermediate between those of their parents (*Kew Bull.*, **14**, No. 1, 88; 1960). Leaves from four genera, *Forsythia*, *Prunus*, *Rhododendron* and *Ribes*, were investigated, with the following results. No exact intermediates were found in which the hybrid interaction was uncomplicated with some other phenomenon. The simplest type of interaction was an intergrading of characters along the leaf-axis, one parent being partially dominant towards the leaf-base and the other towards the apex, as in *Prunus* × *fontanesiana*. The parental effects were equally balanced in the *Rhododendron* hybrid, but there was positive heterosis on the character of relative leaf-breadth. In the *Ribes* hybrid axial intergrading was complicated by negative heterosis on the lengths of the large basal lobes of the leaf and positive heterosis on the breadths of the subterminal lobes. The *Forsythia* hybrid was found to have a leaf-shape exactly intermediate between the leaves of the simple leaved parent and the terminal leaflets of the other parent which had both simple and trifoliolate leaves.

Experiments on Polarity in Flax Buds

H. F. GULLINE has investigated experimentally the polar development in adventitious buds of flax (*Linum usitatissimum*) and their associated vascular

system in hypocotyls (*Aust. J. Bot.*, **8**, 1; 1960). Buds were induced on portions of epidermal tissue grafted in inverted positions on hypocotyls, and on hypocotyls subjected to reversal of the direction of gravity and electric polarity normally acting on them. The effects of experimental incisions into adventitious meristems, and in close proximity to them, were also studied. The basic pattern of development remained constant under all treatments. These experiments show that the epidermal and cortical cells from which the adventitious buds and their dependent vascular traces develop possess a very strong inherent polarity which could not be varied by extracellular changes of field.

Roosting Preferences of a Whiskered Bat

Investigations by Lord Cranbrook, and outlined in a recent issue of the *Transactions of the Suffolk Naturalists Society* (**2**, Part 4), have shown that a whiskered bat, given the opportunity of roosting in a vertical crevice, a horizontal crevice, a bare wall or hanging freely from the roof, showed a strong preference for the first. In a crevice of varying widths, it chose a place where the tips of the hairs on its back just touched the surface behind it, and it did not appear to mind whether it roosted in darkness or light; touch seemed to be the deciding factor.

Popular Culture and Personal Responsibility

The National Union of Teachers is organizing a conference entitled "Popular Culture and Personal Responsibility", which is to be held at Church House, Westminster, London, S.W.1, during October 26–28. The conference has been divided into eight sessions, including such topics as the growth of communications in modern society, moral and cultural standards, the effects of mass media (with particular reference to young people), how the mass media are being used, the responsibility of the provider, the restrictions of working in the media, and the approach and attitude to the mass media. Further information can be obtained from the Public Relations Department, National Union of Teachers, Hamilton House, Mabledon Place, London, W.C.1.

University News: London

Dr. J. S. Rowlinson, senior lecturer in the University of Manchester, has been appointed to the University chair of chemical technology tenable at the Imperial College of Science and Technology.

Among those appointed to readerships are: Dr. R. J. Goldacre (cell physiology), tenable at the Institute of Cancer Research, Royal Cancer Hospital; Mr. M. J. Way, at present principal scientific officer at Rothamsted Experimental Station (applied entomology), tenable at the Imperial College of Science and Technology.

College of Aeronautics, Cranfield

Among the recent appointments to lectureships at the College of Aeronautics are Mr. A. Rothwell (aircraft design) and Dr. A. C. Smith (mathematics).

Announcements

Mr. H. Stanesby has been appointed deputy director of research at the General Post Office. He is at present staff engineer in the Radio Planning and Provision Branch of the General Post Office Engineering Department. Mr. Stanesby has been closely connected with the work of the Institution of Electrical Engineers, and was chairman of the Radio and Telecommunications Section in 1955–56. In 1953 he was awarded the Insignia Award for Technology of the City and Guilds of London for his work in telecommunications.

Dr. D. C. Garratt has been elected chairman of the British Pharmaceutical Conference for 1960–61 and will preside at the meeting of the Conference to be held in Portsmouth during the week beginning September 18, 1961. Dr. Garratt is chief analyst to Boots Pure Drug Co., Ltd., Nottingham; he is also a vice-president of the council of the Society for Analytical Chemistry, and is chairman of its Analytical Methods Committee.

The Palæontographical Society has appointed Messrs. Bailey Bros. and Swinfen as distributing agents for its publications; inquiries about these publications should be sent to Bailey Bros. and Swinfen, Ltd., Hyde House, West Central Street, London, W.C.1.

The Department of Chemical Technology of the Bradford Institute of Technology is holding a special short course on "Recent Developments in Plastics and Polymer Chemistry" during October 28–29. Further information can be obtained from Dr. W. R. Moore, Department of Chemical Technology, Bradford Institute of Technology, Bradford, 7. The fee for the course is £2 5*s*. 0*d*.

The American Association for the Advancement of Science will sponsor a symposium on the Sciences in Communist China during its annual meeting in New York City on December 26–27, under a grant from the National Science Foundation. Preliminary plans call for review papers delivered by eminent American and international specialists on Communist China. Publication of these papers as a monograph is contemplated for the spring of 1961.

Prof. A. R. Ubbelohde (Imperial College of Science and Technology) will deliver the fifth Charles Tennant Memorial Lecture entitled "Melting and Freezing" in the Royal College of Science and Technology, George Street, Glasgow, on November 4. Further information can be obtained from the Society of Chemical Industry, 14 Belgrave Square, London, S.W.1.

The sixth congress on Theoretical and Applied Mechanics and a symposium on High Speed Computation Methods and Machines are being organized by the Indian Society of Theoretical and Applied Mechanics at the University of Delhi during December 23–26. The symposium is being jointly sponsored with the IBM International. Persons wishing to be present at either or both functions should contact Dr. B. R. Seth, Indian Institute of Technology, Kharagpur, India, from whom further information can also be obtained.

Errata. In the article entitled "Evolution of Nuclear Power Plant Design", by Sir Christopher Hinton, in *Nature* of September 24, on p. 1065, col. 2, line 13, the size of uranium rods is wrongly given as 17 in. in diameter; this should be 1 in.; on p. 1067, col. 2, par. 3, line 5, for "reaction" read "reactor".

THE AGRICULTURAL RESEARCH COUNCIL

IN 1958–59 the Agricultural Research Council administered a grant in aid of more than £4 million. Most of this money has been used for established research programmes, since less than half a million pounds was allocated to capital expenditure such as new buildings and the purchase of special equipment. The large expansion programme, embarked on soon after the War, which resulted in the establishment of a number of new institutes as well as extensions to existing institutes, now appears to be nearing its end. There is still one major gap in the field to be covered, and this is referred to in the recent report*, namely, a meat research institute, for which there is a very urgent need. Meat in all its forms is the most valuable commodity coming off British farms, and evaluation of quality is still a very subjective process. We have no thoroughly reliable basis for meat-grading schemes, and there is no real definition of meat quality to provide standards for selection work. Performance and progeny testing of carcass animals is in urgent need of such standards. It is to be hoped that there will be no further delay in the setting up of this institute.

Altogether there are reports from more than forty institutes and research units which are wholly or partly financed by the Council. At one end of the scale there are the giants like Rothamsted with ninety names on the professional staff list, and at the other there are the research units, usually centred at a university, with as few as three or four people working under the direction of a member of the university whose initiative and academic ability have earned the reward of such assistance.

Such units have a continuity usually determined by the duration of the director's university appointment, but this does not generally apply to the special grants which the Council makes to universities, colleges and similar organizations. These are normally limited to three years. A perusal of the list of projects suggests that it is not feasible to organize and complete many of these research tasks in a period so short as three years. Naturally the Council has to safeguard its funds, but it is very important for it to remember that university research cannot prosper if it is on a hand-to-mouth basis so far as finance and personnel are concerned. The quality of the staff the Council recruits, especially in respect of graduates in basic sciences who are in demand from other industries, will depend very much on the kind of research that is being undertaken in the departments that train these recruits. This problem is likely to be aggravated as the 'bulge' descends on the universities, to reduce even further the opportunities university teachers have for personal research.

The reports from the individual institutes are brief and give little information beyond the range of the work that is being undertaken. One cannot, therefore, judge the emphasis that is being placed on the various lines, or estimate the progress that is being made. By way of compensation there are some excellent reviews on several aspects of research in Great Britain along the lines followed in the previous year's report. Because these reviews have a perspective, they are really more valuable than the strictly factual routine report. An outstanding review is that covering foot-and-mouth disease. One sees that the scope of the work being undertaken at the Pirbright Institute has very much more than local implications, and illustrates very effectively the importance of the Council's work to the world at large.

Following the same line of thought, it is heartening to see a reference to the secondment of research workers to countries which have recently achieved self-government, in order to fill the scientific gap created by the retirement of expatriate staff. Countries like Ghana and the Sudan urgently need help, and they are unlikely to obtain it by offering short-term contracts, for there is no certainty that the appointee will get suitable employment when the contract terminates. If, however, he knows that his post is open for him on his return, and that a colleague will replace him abroad to maintain the continuity of his work, then a very different atmosphere is created.

Unfortunately, if we in Britain do not provide this help it will be obtained from other countries, to the detriment of our relations with the emergent countries. This is well evidenced by what is happening in the Sudan. The need for this help exists not only in research but also in teaching, especially at the university level, and also in the provision of technical assistance. It is to be hoped that these initial steps are the fore-runner to a more comprehensive scheme of assistance to countries that need our help.

M. McG. COOPER

* Report of the Agricultural Research Council, 1958–59. Pp. 213. (Cmnd. 1069.) (London: H.M. Stationery Office, 1960.) 9s. net.

THE BACTERIAL GENERA *PSEUDOMONAS* AND *ACHROMOBACTER*

THE summer conference of the Society for Applied Bacteriology was held at the University College of North Wales, Bangor, during July 5–7. A paper-reading session occupied the first day, the last two days being devoted to a symposium on "*Pseudomonas*/*Achromobacter*".

The contributions to this session covered a wide field of both fundamental and applied problems concerning these two groups of bacteria, but the themes which recurred and which promoted most discussion were differentiation and classification. As interests varied widely from the bacterial flora of meat, eggs, fish, water and soil to human and plant diseases, there were wide contrasts of approach and this led to useful exchanges of ideas.

In an introduction to the symposium, Dr. M. Ingram (Low Temperature Research Station, Cambridge) traced the history of the two genera through

the various editions of Bergey's "Manual of Determinative Bacteriology". He showed how over the years there has been a change of emphasis with regard to the importance of flagellation and pigmentation in the definitions of *Pseudomonas* and *Achromobacter*. Some species previously included in the latter genus have now been transferred to the former because of their polar flagellation; previously they had been excluded from the genus *Pseudomonas* because they showed no evidence of pigmentation. Even now that these two genera have been more clearly defined, reconsideration of the placing of some species seems called for. Dr. Ingram made it clear that he in no way wished to decry the value of the "Manual", and this point was later taken up in discussion when the meeting was reminded that the value of the contents of Bergey's "Manual" depends on the standard of work of the bacteriologists whose published descriptions of bacteria are included in it.

Dr. J. M. Shewan (Torry Research Station, Aberdeen) followed with an account of the methods used in his laboratory for the differentiation and classification of the Gram-negative rods associated with fish and for the broad grouping of *Pseudomonas* isolates. For initial separation, these methods depend on the following tests: colony form, pigment production, Gram's stain, motility and morphology (using phase-contrast microscopy), mode of insertion of flagella, antibiotic sensitivity, the oxidase test (Kovacs' method) and metabolism of glucose (Hugh and Leifson's test). *Pseudomonas* isolates are thus divided into three groups. Group I produce a soluble green pigment, oxidize glucose with the production of 2-keto acid and are generally resistant to terramycin. Group II are non-pigmented, oxidize glucose but are usually sensitive to terramycin. Group III are also non-pigmented but fail to oxidize glucose, giving a negative or alkaline reaction in Hugh and Leifson's test. Gram-negative coccoid rods which are non-motile and non-pigmented are classified as *Achromobacter* or *Alcaligenes* according to whether or not they oxidize glucose, while peritrichous rods are classed as Enterobacteriaceae. Other Gram-negative rods recognized are: *Vibrio*, *Aeromonas*, *Flavobacterium* and *Cytophaga*.

Basically the same procedure is being followed at the Low Temperature Research Station, Cambridge, for the examination of isolates from chilled poultry; in addition, they have found the arginine test useful for distinguishing *Pseudomonas* species from *Achromobacter*.

Prof. J. C. Ayres (Food Technology, Iowa State University) has employed further tests for the differentiation of *Pseudomonas* species isolated from poultry, meat and eggs, namely: growth temperature, gelatin liquefaction, fat hydrolysis, nitrate reduction and citrate and carbohydrate utilization. He groups *Pseudomonas* isolates according to whether they resemble *Ps. fluorescens*, *Ps. geniculata*, or *Ps. fragi*.

For isolates from soil, Dr. A. J. Holding (Edinburgh School of Agriculture) has used a similar range of tests; but he has found that litmus milk reactions and nitrogen utilization tests give useful additional information. Unlike workers in the fish and poultry fields, he classes as *Achromobacter* organisms which are motile and predominantly rod-shaped.

A note of caution was sounded by Dr. K. Klinge (Hygienischbakteriologisches Institut, University of Erlangen) in connexion with undue reliance being placed on individual biochemical tests. He instanced the oxidase test where, using Graby and Hadley's method, he found that the number of strains of *Ps. aeruginosa* giving a positive reaction depend on the medium in which they are grown. A yeast extract medium has proved the most favourable.

Dr. A. M. Paton (Department of Bacteriology, Aberdeen) described media that can be used in the isolation and identification of plant pathogenic bacteria. He has found that a gluconate basal broth is semi-selective and is a useful enrichment medium prior to plating on nutrient agar and 5 per cent sucrose agar. With regard to the latter medium, it is important to realize that production of lævan from sucrose is not restricted to certain plant pathogens but is characteristic also of some saprophytic pseudomonads associated with plants. Other tests for identification of plant pathogens suggested by workers in this field were, phage sensitivity and the ability to produce poly-β-hydroxy-butyric acid, a sudanophilic substance, which is characteristic of certain of the non-fermentative Gram-negative rods.

It was evident both from the main papers and from subsequent discussions that the approach of each worker to questions of differentiation and classification depended on the particular problems encountered in his own field. Some, notably those concerned with fish and flesh, wanted at an early stage to be able to separate the non-pigmented *Pseudomonas* from *Achromobacter*. Dr. Holding indicated a further difficulty with soil isolates; saprophytic *Xanthomonas* strains which have lost their ability to form pigment appear to be indistinguishable from the non-pigmented *Pseudomonas*. In contrast to the workers in the above fields, the medical bacteriologists required tests for the rapid detection of *Ps. aeruginosa* and of relatively few other non-fermentative Gram-negative rods. Dr. Klinge described tests he had used to distinguish *Ps. putida* and *Ps. fluorescens* from *Ps. aeruginosa* and also mentioned *Bacterium antitratum*, which he and some other workers have suggested should be included in the genus *Achromobacter* (this view has been questioned by others). For Dr. K. B. Rogers (Children's Hospital, Birmingham), speed of diagnosis is an urgent necessity, and his aim is to find a means of recognizing *Ps. aeruginosa* within 12 hr. of culturing. Plant bacteriologists also were seeking means of distinguishing the pathogenic from the saprophytic *Pseudomonas* within a reasonable period.

Although differentiation on the basis of a few tests was the primary task of most, many were deeply concerned with the classification of their isolates. Several speakers made a plea that the two should not be confused, though it was recognized that what proved to be a useful scheme for differentiation might also be a logical basis for classification. None appeared entirely satisfied with the present position regarding the two genera, *Pseudomonas* and *Achromobacter*, but there was no move to make drastic changes at this stage. The main need seemed to be for all named species to be very fully described and for them to be available in the culture collections. Prof. Ayres stressed the need for rigid standardization of the constituents of media and other factors such as temperature, age and size of inoculum and growth-medium when characterizing bacteria. He also pointed out that the present system of classification is based to a great extent on unstable characters. A number of workers, in the fields of fish, food and water bacteriology, have been using an electronic

computer to analyse their collected results, applying the theory that, in classification, every feature of an organism should be given equal weight. For this procedure, a minimum of 80 different features was quoted as necessary.

Apart from problems of differentiation and classification, considerable attention was paid to the ecology and development of *Pseudomonas* and *Achromobacter*. Workers at the Torry Research Station have found that, while on fresh fish *Achromobacter* species are present in rather larger numbers than *Pseudomonas* species, after about 15 days on ice the latter become dominant. Many of them are dependent on sea-water and can multiply at 0° C. Similarly, it was found at the Low Temperature Research Station that non-pigmented *Pseudomonas* species become dominant on stored chilled poultry. They are associated with 'putrid' off-flavours compared with 'fruity' ones produced by *Achromobacter*. Prof. Ayres has also found that on raw flesh the dominant organisms are coccoid, motile, aerobic and non-pigmented. On eggs, he has encountered various *Alcaligenes* and *Pseudomonas* species, but only a small proportion can penetrate the inner membrane and cause spoliation. Dr. J. Brooks gave an account of factors which limit bacterial multiplication in egg-white; they included lysozyme, *p*H and the inavailability of protein and of iron, biotin and probably other trace elements. He has found that, so far as penetration of the egg membrane is concerned, age is an important factor, and by the twelfth day entry by infecting organisms is much more rapid.

A description of the distribution of Gram-negative organisms, including *Pseudomonas* species, in freshwater, by Miss Vera G. Collins (Freshwater Biological Association, Windermere) threw light on the importance of layering effects on the distribution of bacteria in lakes, while the final paper of the symposium, by Dr. Rogers, showed how a single species, *Ps. aeruginosa*, could persist in and heavily infect surgical apparatus which could not be sterilized by heat. This organism proved highly resistant to the non-corrosive chemical disinfectants and the use of infected apparatus could lead to severe and even fatal infections in children. From the discussion which followed, it appeared that dairy cows have become more liable to infection by this organism in recent years.

Two remaining papers dealt with fundamental approaches to two distinct problems. The first was by Prof. J. de Ley (Microbiology Laboratory, State University, Ghent), who gave a clear and convincing account of the carbohydrate metabolism and localization of enzymes in micro-organisms related to *Pseudomonas*. During the discussion which followed, there was a plea that bacteriologists and the biochemists should work more closely together in the future. It was the feeling of several workers that knowledge of metabolic pathways is of fundamental importance in the classification of bacteria. In the second paper, Dr. A. M. Paton (Department of Bacteriology, Aberdeen), discussed the role of *Pseudomonas* in plant disease. He welcomed the recent increase in knowledge of the metabolism of members of this genus, though as yet the results referred to relatively few species and their general validity is still unknown. After referring to various fundamental problems concerning plant/*Pseudomonas* relationships, he went on to describe the use of plant tissues embedded in nutrient agar for the investigation of pathogenicity mechanisms. He thought that, until more is known of such mechanisms, progress in plant disease control will be limited.

The papers read at the symposium and also contributions to the discussion on "Methods of Isolation and Differential Techniques" will be published in full in the *Journal of Applied Bacteriology*.

EVE BILLING

PHYSIOLOGY OF THE BANANA PLANT

COMPREHENSIVE investigations of the physiology of the banana plant are now being undertaken by Prof. F. C. Steward and colleagues in Cornell University. Of the three parts which have now appeared (*Ann. Bot.*, N.S., **24**, 83, 117, 147; 1960) Part 1 deals with the detection and estimation of biochemical substances in the banana plant, in particular, the nitrogenous constituents of alcohol-soluble extracts, and keto-acids and non-volatile organic acids. The classes of nitrogen compounds to which attention is given are the amino-acids and their amides, certain volatile amines, ureido and guanido compounds, and certain indole compounds. In view of the large amount present in fruit of the Gros Michel variety, free histidine was isolated and critically identified. The use of various reagents and tests for these various classes of substances on paper chromatograms are described.

The range of substances which have been detected by these means in the banana leaf, leaf-sheath, fruit (pulp and peel) and stem exudate is shown by their location on paper chromatograms. Evidence is presented which indicates qualitatively the degree of prominence of the different substances in the tissues or extracts which have been examined.

Part 2 relates to factors which affect the nitrogen compounds of the fruit of several varieties subsequent to harvesting. In Gros Michel from Honduras, the amides (asparagine and glutamine) and histidine are of special importance in the soluble nitrogen fraction of the fruit. Prominent changes occur in the nitrogen compounds as the fruit ripens, and these are shown to be markedly influenced by the conditions that obtain during the development of the fruit. The nitrogenous composition of the fruit (Gros Michel) is markedly affected by the season of the year at which it forms; this affects the balance between the amides (asparagine and glutamine) and histidine, and also influences the post-harvest metabolic behaviour in ways which are described. While the total protein of the fruit is relatively stable in its amino-acid composition, certain differences were noted which seem to characterize the Gros Michel as it grows in Central America, and some shifts in the amino-acid balance in the protein seem to occur with ripening. It was also ascertained that the very young inflorescence is at first relatively rich in soluble nitrogen compounds.

After the early phase of growth, involving cell division, has elapsed, the fruit utilizes its soluble

nitrogen in synthesis faster than it is supplied to the fruit, so the soluble nitrogen complement falls to a low level; thereafter it increases again to a new higher level at harvest. The composition of the soluble nitrogen fraction as stored at harvest is quite different from that which obtains in the inflorescence in which the fruit is being initiated. The latter emphasizes the amides (asparagine and glutamine) and histidine; the former emphasizes alanine and glutamine as the only prominent amide.

In Part 3, nitrogen compounds of the leaves are considered. The materials analysed were obtained from plants grown in sand cultures either supplied with full nutrient solution or with solutions deficient in each of the several major nutrient elements. The growth was continued long enough to establish the conditions characteristic of these nutrient treatments. Extracts of leaves, sampled according to their position on the axis, have been made from plants grown in full nutrient solution; these extracts have been examined by chromatographic methods to detect and determine the various nitrogen compounds they contain.

The relative proportions of the soluble nitrogen compounds of the banana leaf are quite different from those of the fruit and, in response to the deficiency of specified mineral nutrients, both the total amount and the relative composition of the soluble nitrogen fraction are greatly affected. The results are interpreted in terms of the more active accumulation of soluble compounds in young leaves and the maintenance of a low amide (glutamine)-level in leaves engaged in protein synthesis. The accumulation of soluble nitrogen compounds when growth and synthesis are arrested, and the relative accumulation of a specified number of nitrogenous substances, due to the lack of a nutrient element, indicate that metabolic blocks in reaction pathways occur.

THE NUCLEUS OF THE ANDROMEDA NEBULA

IN 1959 the electronic camera developed by Lallemand and Duchesne in Paris was taken to the Lick Observatory on Mount Hamilton, California, and installed at the focus of the coudé spectrograph of the 120-in. reflector. Among the objects chosen for observation was the nucleus of the Great Nebula in Andromeda, *M* 31. The nucleus appeared on the slit as almost stellar, the inner parts of the nebula which are normally so conspicuous on photographs being invisible.

The results of the observations have been reported by A. Lallemand, M. Duchesne and M. F. Walker (*Pub. Astron. Soc. Pacific*, **72**, 76; 1960). The first spectrogram of the nucleus was made in very good seeing when a stellar image was about half a second of arc in diameter, and the image of the nucleus was of diameter 4·4″. It showed at once that the *H* and *K* lines of Ca II, which were the only ones clearly visible, were markedly inclined through the nucleus. Subsequent observations confirmed this. The observations have been interpreted on the assumption that the inclination of the lines is produced by the rotation of the nucleus, and, when converted to the equivalent velocities in circular orbits, the measures yield a maximum circular velocity of 87 km./sec. at a distance of 2·2″ from the centre of the nucleus. Beyond this distance the velocity appears to decrease, a velocity of 42 km./sec. being measured at 4·2″ from the centre.

Previous observations of the rotation of *M* 31 refer to points farther from the centre, the innermost point being at 25″ from the centre. The velocity apparently has a minimum somewhere in the region of 10″ from the centre, a maximum at 3′, another minimum at 9′, and an increasing velocity for greater distances. If one assumes that the nucleus is spherical, of radius 2·2′, and that it rotates as a solid body with a maximum circular velocity of 87 km./sec., its properties may be calculated. Its radius is 7·4 parsecs, its mass $1{\cdot}3 \times 10^7$ solar masses, its average density $1{\cdot}5 \times 10^3$ solar masses per cubic parsec and its rotation period $5{\cdot}2 \times 10^5$ years. Its mass-to-luminosity ratio is about 3·6 in solar units. The nucleus is therefore similar in size to a giant globular cluster, but one hundred times more massive and twenty times as dense and as luminous. It rotates one to two orders of magnitude faster than the rest of *M* 31. The results indicate that the nucleus is a separate dynamical entity, although spectroscopic evidence suggests that its stellar composition is like that of the surrounding central regions.

RESULTS OF THE OCCULTATION OF REGULUS BY VENUS, JULY 7, 1959

By Dr. G. de VAUCOULEURS and Dr. D. H. MENZEL
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1. Ingress and Egress Observations

TABLE 1 gives the times T_1, T_2 of the half-intensity phase, $\Delta m = 0{\cdot}75$ mag., at ingress and egress derived from visual, photographic and photoelectric observations secured by members of the Harvard College Observatory (stations 1*a*, 2, 3, 4, 5, 7) and Smithsonian Astrophysical Observatory (stations 1*b*, 6) expeditions. Rain and clouds prevented observations at two other stations (Castel Gandolfo, Asiago). Additional observations (stations (8–24) were received by letter or taken from published sources. Station co-ordinates, observers and sources are listed in Table 2. Published observations

Table 1. Observed Times of Ingress and Egress at Half-intensity*

No.	Station	T_1	T_2	T_2-T_1	T_m	$(O-C)_1$	$(O-C)_2$	Sec β	Obs.†
1	Madrid	19m. 34·2s.	30m. 41·8s.	667·6s.	25m. 08·0s.	+ 0·87s.	+ 0·11s.	1·274	2V
2	Le Houga	20 10·2	30 28·5	618·3	25 19·5	+ 0·33	+ 2·91	1·382	V + Pe
3	Merate	21 30·5	30 26·5	536·0	25 58·5	+ 1·03	− 1·89	1·578	Pg
4	Catania	21 49·0	32 05·0	616·:	26 57·:	− 0·81	(+ 6·21)	1·400	V
5	Beirut	24 41·2	33 03·8	502·6	28 52·5	− 0·09	− 1·59	1·687	V + Pg
6	Shiraz	27 10·5	—	—	—	− 2·20	—	2·150	2V
7	Bloemfontein	24 52·2	38 49·7	837·5	31 51·0	+ 0·47	− 0·46	1·018	V + Pe
8	Pretoria	24 51·8	38 57·2	845·4	31 54·5	+ 0·33	− 0·35	1·008	V
9	Hartebespoort	24 47·8	38 55·2	847·4	31 51·5	− 1·83	− 0·25	1·009	2V
10	Utrecht	21 23·5	29 21·5	478·0	25 22·5	− 5·53	+ 2·93	1·806	2V
11	Copenhagen	22 47·5	28 31·5	344·1	25 39·5	− 3·15	− 4·28	2·453	V
12	Uppsala	24 27·5	27 12·5	165·:	25 50·:	− 0·50	+ 1·86	5·179	V
13	Johanneshov	24 26·5	27 20·5	174·:	25 53·5	+ 2·09	− 0·32	4·793	V
14	Munich	21 56·5	—	—	—	− 0·94	—	1·739	V
15	Mulhouse	21 20·5	30 02·0	521·5	25 41·2	− 1·41	− 2·90	1·624	V
16	Armagh	20 22·7	—	—	—	+ 0·08	—	1·696	V
17	Dunsink	20 19·0	28 52·3	513·3	24 35·7	+ 1·03	+ 1·13	1·651	V
18	Ascot	20 44·0	—	—	—	+ 0·75	—	1·648	V
19	Uccle	21 17·5	29 29·0	491·5	25 23·2	+ 4·45	− 3·82	1·714	V
20	Leyden	21 18·7	—	—	—	− 5·49	—	1·795	V
21	Zurich	21 28·8	30 08·4	519·6	25 48·6	− 0·96	− 2·00	1·632	V
22	Innsbruck	21 39·5	30 16·0	516·5	25 57·7	(−12·09)	+ 1·06	1·688	V
23	Bucharest	23 47·0	30 56·7	429·7	27 21·8	+ 0·50	+ 2·04	1·984	V
24	(South Africa)	24 50·8	38 57·0	846·2	31 53·9	− 0·70	− 0·60	1·008	2V

* Interpolated and corrected for personal equation where required. All times are U.T. 14 hr. +.
† *V*, visual; *Pg*, cinematographic; *Pe*, photoelectric (ingress only).

Table 2. Stations Co-ordinates, Instruments, Observers and Sources

No.	Station	Longitude	Latitude	Altitude (m.)	Instrument cm.	×	Observers	Sources
1*a*	Madrid	+ 03° 41′ 15″	+ 40° 24′ 30″	650	15*r*	250	H. C. Ingrao	H.C.O.
1*b*	Madrid				12*r*	—	J. A. Hynek	S.A.O.
2*a*	Le Houga	+ 00° 11′ 15″	+ 43° 46′ 40″	140	20*r*	*Pe*	G. de Vaucouleurs	H.C.O.
2*b*	Le Houga				20*r*	300	A. de Vaucouleurs	H.C.O.
3	Merate	− 09° 25′ 42″	+ 45° 41′ 54″	334	49*r*	*Pg*	R. O. Doyle	H.C.O.
							H. W. Ocker	Boeing Co.
4	Catania	− 15° 05′ 11″	+ 37° 30′ 13″	049	15*r*	—	D. H. Menzel	H.C.O.
					30*r*	—	M. G. Fracastoro	
5	Beirut	− 35° 28′ 11″	+ 33° 54′ 22″	038	30*r*	*Pg*	O. Gingerich	H.C.O.
					12*r*	—	M. Lesley	
6*a*	Shiraz	− 52° 31′ 34″	+ 29° 38′ 40″	1,596	18*R*	110	C. F. Capen	S.A.O.
6*b*	Shiraz				15*R*	80	V. W. Capen	
7*a*	Bloemfontein	− 26° 24′ 21″	− 29° 02′ 18″	1,387	33*r*	*Pe*	J. Anderson	Boyden (H.C.O.)
							H. Haffner	
7*b*	Bloemfontein				30*R*	—	C. Hoffmeister	I.A.U. circ. 1684
								MNASSA, 18, 97
8	Pretoria	− 28° 13′ 44″	− 25° 47′ 18″	1,542	188*R*	720	A. D. Thackeray	MNASSA, 18, 63
9*a*	Hartebespoort	− 27° 52′ 38″	− 25° 46′ 22″	1,220	15*r*	—	H. C. Lagerweij	MNASSA, 18, 52
							J. A. Bruwer	
9*b*	Hartebespoort				20*r*	—	Th. Walraven	Union Obs. Annex
							J. W. Muller	Comm. by W. Finsen
10*a*	Utrecht	− 05° 07′ 48″	+ 52° 05′ 10″	012	25*r*	—	(Staff)	Sterrewacht Sonneborgh
10*b*	Utrecht				40*R*	—	(Staff)	Comm. by M. Minnaert
11	Copenhagen	− 12° 27′ 11″	+ 55° 42′ 13″	014	11*r*	180	J. Trier	I.A.U. Circ. 1683
							P. Darnell	
12	Uppsala	− 17° 37′ 30″	+ 59° 51′ 29″	021	36*r*	200	A. Walenquist	Comm. by G. Larsson-Leander
							L. Peterson	
13	Johanneshov	− 18° 06′ :	+ 59° 18′ :	—	15*R*	290	L. Dahlmark	Comm. by G. Larsson-Leander
14	Munich	− 11° 36′ 30″	+ 48° 08′ 45″	529	28*r*	250	W. D. Heintz	Mitt. St. München, I, Nr. 9
15	Mulhouse	− 07° 18′ 11″	+ 47° 44′ 39″	—	20*R*	210	H. Kern	L'Astron., 73, 383
16	Armagh	+ 06° 38′ 51″	+ 54° 21′ 13″	062	25*r*	100	Papworth	Irish A.J., 5, 212
17	Dunsink	+ 06° 20′ 16″	+ 53° 23′ 13″	086	30*r*	—	M. A. Ellison	Irish A.J., 5, 212
18	Ascot	+ 00° 38′ 20″	+ 51° 24′ 38″	—	15*r* ?	—	R. L. Waterfield	I.A.U. Circ. 1684
19	Uccle	− 04° 21′ 30″	+ 50° 47′ 55″	105	13*r*	109	A. Velghe	I.A.U. Circ. 1684
20	Leyden	− 04° 29′ 02″	+ 52° 09′ 20″	006	?	—	A. G. Jansen	I.A.U. Circ. 1685
21	Zurich	− 08° 32′ 50″	+ 47° 22′ 02″	469	30*r*	85	R. A. Naef	I.A.U. Circ. 1684; Orion, No. 66
22	Innsbruck	− 11° 22′ 51″	+ 47° 16′ 08″	605	18*r*	—	O Schneider	I.A.U. Circ. 1688
							J. Fuchs	
23	Bucharest	− 26° 05′ 48″	+ 44° 24′ 50″	083	?	—	C. Christescu	I.A.U. Circ. 1683
24	(South Africa)	− 28° 14′ 39″	− 25° 46′ 46″	1,440	30*R*	305	N. M. Hoogenhout	MNASSA, 18, 52, 62
					15*R*	77	P. G. van der Byl	MNASSA, 18, 52, 62

r, refractor; *R*, reflector; ×, magnification (visual observation); *Pg*, photographic; *Pe*, photoelectric.

at more than twenty other stations were found too discordant or incomplete for inclusion in the final solution. The 'observed' times in Table 1, when derived from visual observations, are interpolated to $\Delta m = 0{\cdot}75$ mag. with the help of the theoretical light curve derived from the photoelectric observations of ingress at stations 2 and 7 and are corrected where required for a personal equation of 0·2–0·3 sec. on the average.

2. Geometric Results

The observed mid-eclipse times $T_m = \frac{1}{2}(T_1 + T_2)$ and durations $D = T_2 - T_1$ were compared with the values computed from the apparent co-ordinates of Venus and of α Leonis (α = 10h. 06m. 12·193s., $\delta = +12°\ 09'\ 56{\cdot}424''$) derived from the American ephemeris data. The ephemeris radius and parallax of Venus were $R_V = 14{\cdot}25''$, $R_E = 14{\cdot}91''$ for a dis-

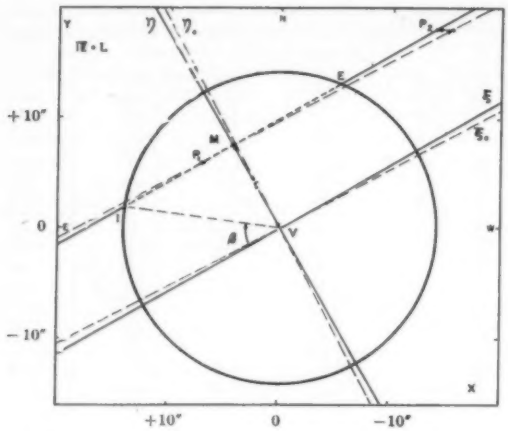

Fig. 1. Projection on plane tangent to celestial sphere of apparent motion of Regulus with respect to Venus. P_1, P_2, apparent positions at 14h. 24m. and 14h. 36m. U.T. *I*, ingress; *E*, egress; *M*, mid-occultation point ($MV = r$). Dashed lines, geocentric paths

tance $\Delta = 0{\cdot}59005$ A.U. ($\sigma_1 = 8{\cdot}41''$, $\pi\odot = 8{\cdot}80''$). The speed of the apparent motion of Venus was approximately $\dot{s} = 0{\cdot}0339''$ per sec. of time in position angle $\theta = 118°\ 37'$, the exact values varying slightly from station to station.

The mean *O–C* for the mid-eclipse time from nineteen stations, $\Delta t = T_m$ (observed U.T.) $- T_m$ (computed E.T.) $= -\ 28{\cdot}1$s. $\pm\ 0{\cdot}2$s.*, can be interpreted as a determination of Ephemeris Time from the motion of Venus in longitude. A provisional value for 1959·5 derived at the U.S. Naval Observatory from observations of the Moon is $\Delta t_{\mathbb{C}} = -\ 32{\cdot}4$s. (Markowitz, W., personal communication). The 4·3 sec. difference between the two values is well within the possible errors in the star position and in the Venus ephemeris (several tenths of a second of arc).

If ΔR, Δr are the corrections to the ephemeris values of the radius of Venus R_e and of the minimum distance r_e between the star and the centre of the disk (Fig. 1), the difference *O–C* between the observed and computed durations $D = T_2 - T_1$ is to the first order:

$$\dot{s}\ (D_o - D_c) \cos \beta_e = \Delta R - \Delta r \sin \beta_e \qquad (1)$$

with $\sin \beta_e = r_e/R_e$. Starting from first-approximation values of ΔR, Δr determined graphically, a least-squares solution for sixteen stations leads to:

$$\Delta R = +\ 0{\cdot}150'' \pm 0{\cdot}005'', \quad \Delta r = +\ 0{\cdot}315'' \pm 0{\cdot}010''$$

The corrections Δt and Δr when applied to the ephemeris data give the following values for the observed differential geocentric co-ordinates (α Leo − Venus) on July 7, 1959, at 14h. 24m. 00s. Universal Time:

$$\Delta\alpha \cos \delta = +\ 9{\cdot}110'' \pm 0{\cdot}010''$$
$$\Delta\delta = -\ 0{\cdot}334'' \pm 0{\cdot}010''$$

The final *O–C* of the ingress and egress times computed with these corrected co-ordinates and the observed planetary radius $R_0 = R_e + \Delta R = 14{\cdot}400''$ are listed in Table 1 and plotted in Fig. 2 as a function of $\sin \beta = r/R$. All residuals are less than ± 6s., except at ingress in station 22 (rejected), and at egress in station 4, where observation was interfered with by a thick haze; the average deviation for the other stations is ± 1·6s. at ingress ($n = 23$), ± 1·7s. at egress ($n = 18$). The mean *O–C* is − 0·51s. ± 0·22s. ($n = 23$) at ingress and − 0·36s. ± 0·27s. ($n = 18$) at egress. However, the photoelectric light curves of ingress at Le Houga and Bloemfontein (section 3) give $(O–C)_1 = +\ 0{\cdot}4$s. ± 0·3s. and + 0·3s. ± 0·4s., respectively, and the excellent visual observations at Pretoria $(O–C)_{1,2} = 0{\cdot}0$s. ± 0·3s. No residual correction is indicated.

The observed radius $R_0 = 14{\cdot}400''$ does not refer to the cloud-level, but to a much higher level in the upper atmosphere where the differential refraction effect amounts to 0·75 mag. This radius must be further corrected for the total refraction $\omega_0 = 0{\cdot}016''$ at this level (section 3), since the observed durations are shortened by refraction (Fig. 3). The corresponding radius at unit distance then is:

$$\sigma_1(z_0) = (R_0 + \omega_0) \times \Delta = 8{\cdot}506'' \pm 0{\cdot}003''$$

This value demonstrates that the numerous filar micrometer determinations of the semi-diameter of Venus in excess of 8·5″ are in error; the best measurements with double-image micrometers during transits or with the filar micrometer on the narrow crescent phases give $\sigma_1 = 8{\cdot}41'' \pm 0{\cdot}01''$ in agreement with the ephemeris value. The altitude of the occulting shell (at half intensity) then is:

$$\sigma_1\ (z_0) - \sigma_1\ (z_c) = 0{\cdot}096'' \pm 0{\cdot}011''$$

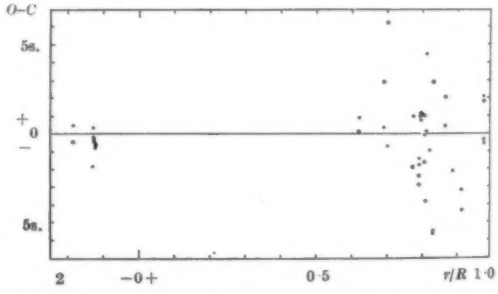

Fig. 2. Residuals *O–C* of ingress (●) and egress (○) times at half-intensity for corrected elements versus $\sin \beta = r/R$

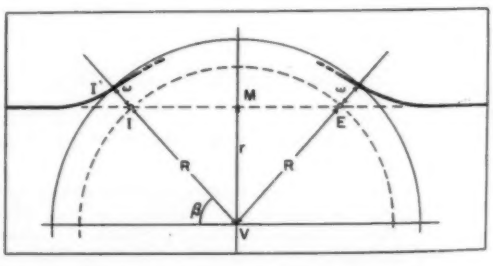

Fig. 3. Effect of atmospheric refraction on duration of occultation. At the times of apparent ingress in *I*, and egress in *E*, the geometric positions of the star are in *I* and *E*; the apparent radius $R = VI = VE$ corresponding to the observed duration $D = T_2 - T_1$ of the occultation is less than the true radius $R' = VI' = VE'$ of the occulting shell; the difference $\omega = II' = EE'$ is the total deviation by refraction

* All errors quoted are p.e.

or :

$$z_0 - z_c = 70 \pm 8 \text{ km.}$$

above the cloud-level.

3. Photometric Results

The fading phase of ingress at the dark limb was successfully observed photoelectrically at the Péridier Observatory, Le Houga, France (station 2), and at the Boyden Observatory, Bloemfontein, South Africa (station 7). The unsmoothed readings at 0·24-sec. intervals are compared in Figs. 4 and 5 with theoretical light curves computed from the classical theory of extinction by differential refraction in a homogeneous atmosphere[1,2]. It was not found possible to fit the observations with a constant scale height $H = RT/mg$, that is, a homogeneous isothermal atmosphere; both curves indicate a small, but definite, increase of H with altitude. In the observable range $| z - z_0 | < 20$ km., the scale height may be written :

$$H(z) = H(z_0) \left[1 + \frac{1}{H} \frac{\partial H}{\partial z} (z - z_0) \right]$$

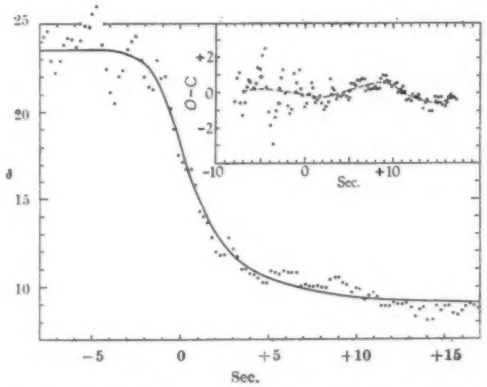

Fig. 4. Photoelectric observations of ingress at the Péridier Observatory, Le Houga, France, compared with theoretical curve for adopted scale height. Inset : residuals, note damping of scintillation during fading phase

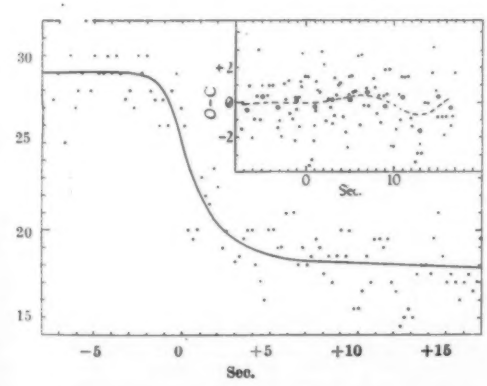

Fig. 5. Photoelectric observations of ingress at Boyden Observatory, Bloemfontein, South Africa, compared with theoretical light curve for adopted scale-height. Inset, residuals

with :

$$H(z_0) = 6{\cdot}8 \pm 0{\cdot}2 \text{ km.}$$

(corresponding to a total refraction angle $\omega_0 = H/\Delta = 0{\cdot}0160''$)[2] and :

$$\frac{1}{H} \frac{\partial H}{\partial z} = + 0{\cdot}010 \pm 0{\cdot}002 \text{ km.}^{-1}$$

The residuals are shown in the insets ; the variation of H with height, although small, seems real, since it was not possible to secure a satisfactory fit with a constant value. Note that because of the different geometry of the occultation in France (sec $\beta = 1{\cdot}38$) and in South Africa (sec $\beta = 1{\cdot}02$), the actual time-scales for fading are notably different in the two stations ; the satisfactory fit of both curves by a single set of parameters is therefore a significant check.

With the acceleration of gravity $g(z_0) = 860$ cm. sec.$^{-2}$, we have :

$$\frac{T}{m} = 1{\cdot}03\, H$$

or, for $H = H(z_0)$:

$$T(z_0) = 7{\cdot}0\, \overline{m}$$

For the plausible value $m = 42{\cdot}5$, corresponding to the assumed volume composition carbon dioxide = 0·90, nitrogen = 0·09, argon + others = 0·01 :

$$T(z_0) = 297 \pm 10° \text{ K.}$$

(280° K. for nitrogen/carbon dioxide = 1, $m = 36$; 308° K. for nitrogen/carbon dioxide = 0, $\overline{m} = 44$).

Further, if we assume that $\partial \overline{m}/\partial z = 0$ (that is, no photodissociation of carbon dioxide or nitrogen in the range $z_0 \pm 20$ km.), the value of $\partial H/\partial z$ implies :

$$\frac{\partial T}{\partial z} \simeq + 3° \text{ km.}^{-1}$$

or a range of variation from 240° K. near $z - z_c = 50$ km. to 360° K. near $z - z_c = 90$ km.

The total pressure at altitude z_0 is :

$$p(z_0) = 2{\cdot}6 \pm 0{\cdot}13 \text{ dynes cm.}^{-2} ;$$

it is practically independent of the adopted mixing ratio nitrogen/carbon dioxide. The corresponding mass and reduced thickness above z_0 are :

$$m(z_0) = 3{\cdot}0 \times 10^{-3} \text{ gm.}$$
$$h(z_0) = 1{\cdot}7 \text{ cm. N.T.P.}$$

The reduced thickness of the atmosphere *along* the light path for the grazing ray at z_0 is :

$$X(z_0) = 1{\cdot}3 \text{ m.}$$

Absorption effects by known or suspected atmospheric gases must therefore be entirely negligible and the observed light curve can be safely interpreted in terms of pure differential refraction effects.

Given the photometric curve for a central occultation (Fig. 6) and the corrected elements of section 2, it is easy to compute the magnitude loss $\Delta m = -2{\cdot}5 \log I/I_0$ at the observed instants of disappearance T_D

Table 3. Magnitude Differences at Disappearance and Reappearance

No.	Station	T_D	$\frac{O-C}{\sec\beta}$	Δm_D	T_R	$\frac{O-C}{\sec\beta}$	Δm_R	$\frac{D-R}{\text{mag.}}$	Tel. cm.	×	Remarks
1*a*	Madrid	19m. 37·1s.	+ 2·98s.	2·15	30m. 42·3s.	+ 0·47s.	0·50	+ 1·65	15*r*	250	
1*b*	Madrid	36·9	+ 2·83	2·08	41·3	− 0·31	0·94	+ 1·14	12*r*	—	
2*b*	Le Houga	20 13·1	+ 2·31	1·90	30 25·7 ?	+ 0·07	0·72	+ 1·18	20*r*	300	•
3	Merate	21 31·0	+ 0·95	1·30	30 27·5	− 0·57	1·10	+ 0·20	49*r*	*Pg*	•
4	Catania	21 49·7 :	− 0·07	0·70	32 09·7 :	+ 7·79	rej.	+ 0·70	15*r*, 30*r*	—	•
5	Beirut	24 43·5	+ 1·31	1·47	33 05·5 :	+ 0·06	0·72	+ 0·75	12*r*	—	
6*a*	Shiraz	27 14·7	+ 0·93	1·29	—	—	—	+ 1·29	18*R*	105	•
6*b*	Shiraz	14·6	+ 0·88	1·25	—	—	—	+ 1·25	15*R*	80	
7*b*	Bloemfontein	24 59·8 :	+ 7·96	3·17	38 49·3	− 0·88	1·25	+ 1·92	30*R*	—	
8	Pretoria	24 58·3	+ 6·75	3·00	38 54·8	− 2·78	2·03	+ 0·97	188*R*	720	
9*a*	Hartebespoort	24 49·2	− 0·40	0·54	38 55·7	+ 0·20	0·65	− 0·11	15*r*	—	
9*b*	Hartebespoort	49·3	− 0·30	0·59	55·8	+ 0·30	0·59	0·00	20*r*	—	
10*a*	Utrecht	21 31·1	+ 1·16	1·39	29 25·0	+ 3·54	0·00	+ 1·39	25*r*	—	
10*b*	Utrecht	30·9	+ 1·05	1·35	22·2 :	+ 1·99	0·05	+ 1·30	40*R*	—	
11	Copenhagen	22 50·8	+ 0·04	0·79	28 32·6	− 1·30	1·46	− 0·67	11*r*	180	
12	Uppsala	24 29·5 :	+ 0·29	0·94	27 14·5 :	+ 0·75	0·35	+ 0·59	36*r*	200	•
13	Johanneshov	24 33·5 :	+ 1·90	1·64	27 32·5 :	+ 2·44	0·03	+ 1·61	15*R*	290	•
14	Munich	21 58·8	+ 0·81	1·22	—	—	—	—	28*r*	250	
15	Mulhouse	21 22·7	+ 0·49	1·05	30 02·7	− 1·35	1·49	− 0·44	20*R*	210	
16	Armagh	20 25·0	+ 1·42	1·53	—	—	—	—	25*r*	100	
17	Dunsink	20 24·8	+ 4·12	2·46	28 50·8 ?	− 0·24	0·90	+ 1·56	30*r*	—	•
18	Ascot	20 46·3	+ 1·82	1·70	—	—	—	—	15*r* ?	—	
19	Uccle	21 19·8	+ 3·91	2·40	29 29·8	− 1·75	1·67	+ 0·73	13*r*	109	
20	Leyden	21 23·4	− 0·45	0·52	—	—	—	—	?	—	
21	Zurich	21 29·6	− 0·12	0·68	30 09·2	− 0·73	1·16	− 0·48	30*r*	85	•
22	Innsbrück	21 41·8	− 5·81	rej.	30 16·8	+ 1·13	0·20	− 0·20	18*r*	—	
23	Bucharest	23 52·2	+ 2·87	2·10	30 57·6	+ 1·46	0·12	+ 1·98	?	—	
24*a*	(South Africa)	24 53·2	+ 1·69	1·65	38 57·5	− 0·10	0·92	+ 0·73	30*R*	305	

T_D (or T_R), time when last seen at ingress (or first seen at egress), corrected for personal equation; $O-C$, observed T_D (or T_R) minus computed ingress (egress) times at half-intensity T_I (or T_E); Δm, magnitude difference read on standard light curve at time $t = T_I$ (or T_E) + $(O-C)/\sec\beta$; $D-R$, $\Delta m_D - \Delta m_R$.

Station 2*b*: T_R, doubtful at 25·7s., definite at 28·8s. Station 3: T_R, doubtful at 25·5s., definite at 27·5s. Station 17: T_R, doubtful at 50·8s., definite at 53·8s. Station 12: T_D, ± 10s. (clouds). Stations 3, 4, 6*a*, 12 (T_R), 13 (T_D), 16, 21: hazy sky.

and reappearance T_R (Table 3). At both ingress and egress the gain in Δm for large telescopes was only slight; on the average $\Delta m_D = -0{\cdot}5 + 1{\cdot}5 \log A$ at ingress, and $\Delta m_R = -1{\cdot}4 + 1{\cdot}5 \log A$ at egress, if A is the telescope aperture in cm. On the average, $\Delta m(D) - \Delta m(R) = +0{\cdot}9$ mag., that is, the star was not detected at the bright limb until it was more than twice as bright as when last seen at the dark limb. For instruments in the range $A = 10$–40 cm., $\overline{\Delta m_D} \simeq 1{\cdot}5$ mag., $\overline{\Delta m_R} \simeq 0{\cdot}6$ mag. with a dispersion $\sigma \approx \pm 0{\cdot}3$ mag.; in stations with a bright, hazy sky $\Delta m_D \approx 0{\cdot}5$ to $1{\cdot}0$ mag. and $\Delta m_R \approx 0{\cdot}0$ (with T_R occasionally late by 5–10 sec. or more); in stations with good seeing and dark blue sky $\Delta m_D \approx 2{\cdot}0$ to $2{\cdot}5$ mag.

4. Atmospheric Model

A tentative atmospheric model incorporating the occultation results is shown in Fig. 6. The schematic temperature curve is based on the following data:

(1) Surface temperature: $T(0) = 580°$ K. (from micro-wave observations).

(2) Lower stratospheric temperature: $T(z^*) = 285°$ K. (from spectroscopic observations) at an estimated altitude $z^* \approx (580 - 285)/10 = 29{\cdot}5$ km. for an assumed mean adiabatic gradient $\Gamma = -10 \pm 1°$ K. per km.; according to Kuiper, the top of the cloud layer is about $0{\cdot}46\ H$ lower, hence $z_c \approx 27 \pm 5$ km.

(3) Upper stratospheric temperature: $T(z_r) = 235°$ K. (from the radiometric observations) at an estimated altitude $z_r - z_c \approx 40$–50 km. or $z_r \approx 65$–75 km.

(4) Occultation-level temperature: $T(z_0) = 297°$ K. at $z_0 - z_c \approx 70$ km. or $z_0 \approx 95$–100 km.

With this temperature curve the mean temperature between z_0 and z_c is $\bar{T} = 260 \pm 10°$ K. and the mean scale height $\bar{H} = 6{\cdot}0 \pm 0{\cdot}2$ km., so that:

$$\log p(z_c) - \log p(z_0) = (z_0 - z_c)/2{\cdot}30\ \bar{H} = (z_0 - z_c)/13{\cdot}7$$

If we assume for the pressure at the top of the cloud layer the commonly accepted value $p(z_c) = (1{\cdot}6 \pm 0{\cdot}2) \times 10^5$ dyne cm.$^{-2}$, the observed pressure at the occultation-level $p(z_0) = (2{\cdot}6 \pm 0{\cdot}13)$ dyne cm.$^{-2}$ implies an altitude difference:

$$z_0 - z_c = (5{\cdot}20 - 0{\cdot}41) \times 13{\cdot}7 = 65{\cdot}5 \pm 2{\cdot}5 \text{ km.}$$

in good agreement with the observed value 70 ± 8 km. The pressure distribution $p(z)$ corresponding to the temperature curve and the adopted value of $p(z_c)$ is shown in Fig. 7. The surface pressure of the

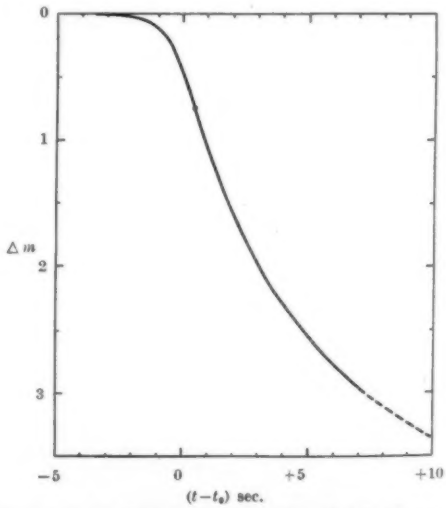

Fig. 6. Standard light curve for central occultation ($\beta = 0$) on July 7, 1959, with $H(z_0) = 6{\cdot}8$ km., $\partial H/\partial z = +0{\cdot}01\ H$

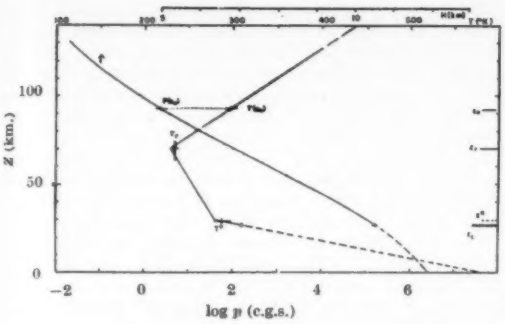

Fig. 7. Atmospheric model incorporating micro-wave, radiometric, spectroscopic and occultation data. Schematic temperature distribution (scale at top) and derived pressure curve for $p(z_c) = 160$ millibars at top of cloud layer ($z_c \approx 27$ km.). The occultation pressure $p(z_0) = 2{\cdot}6$ dyne cm.$^{-2}$ obtains at $z_0 - z_c = 65{\cdot}5$ km., where $T(z_0) = 297°$ K.

model, $p_0 \approx 2{\cdot}5$ atm., uncertain by a factor 2–3, is not directly relevant to the interpretation of the optical data.

If $\sigma_1(z_0) = 8{\cdot}506'' \pm 0{\cdot}003'' = 6{,}165 \pm 2$ km., $z_0 - z_c = 65 \pm 2{\cdot}5$ km. and $z_c = 27 \pm 5$ km., the optical semi-diameter of Venus then is $\sigma_1(z_c) = 8{\cdot}506'' - 0{\cdot}090'' = 8{\cdot}416'' \pm 0{\cdot}005'' = 6{,}100 \pm 4$ km. and the radius of the solid globe $\sigma_1(0) = 8{\cdot}506'' - 0{\cdot}127'' = 8{\cdot}379'' \pm 0{\cdot}008'' = 6{,}089 \pm 6$ km. $= 0{\cdot}955_5 \pm 0{\cdot}001$ ($\delta = 1$).

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[1] Pannekoek, A., *Astr. Nach.*, **164**, No. 3913 (1903).
[2] Fabry, Ch., *J. des. Obs.*, **12**, 1 (1929).

VARIATION WITH TEMPERATURE OF YOUNG'S MODULUS OF POLYCRYSTALLINE GRAPHITE

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IT is well known that at 1,000° C. the elastic moduli of artificial graphites rise with temperature[1,2]. It is also often assumed that this positive variation with temperature exists down to room temperature, although there appear to be no actual measurements to confirm this. Some fairly precise measurements of Young's modulus from 20° to 1,000° C. which we have made indicate that the variation with temperature is not always positive ; there is, in fact, a well-defined minimum in the region of 200° C. This is a result of considerable importance in connexion with work on the structure of polycrystalline graphite artefacts, and is also of fundamental interest in indicating that the basic graphite crystallite probably exhibits a negative temperature coefficient of one or other of its elastic moduli, in conformity with most crystalline materials.

The only other evidence of a negative temperature coefficient for a carbon in this temperature-range appears to be that given by Davidson *et al.*[3] for an artefact heat-treated to 1,400° C. They found a net negative variation of rigidity modulus between 0° and 1,000° C. and continuing up to 1,400° C., but with no indication of this variation becoming positive.

Representative results obtained in the present investigation are shown in Fig. 1. These relate to a nuclear reactor grade carbon stock, heat-treated to final temperatures of 1,200°, 2,100° and 2,600° C. respectively. The modulus was measured in all cases in a direction parallel to the extrusion axis. The measurements were made by an adaptation of the flexural vibration method first described by Forster[3]. The specimens, 4 in. long and $\frac{1}{4}$ in. diameter, were suspended horizontally in a platinum-wound mullite furnace tube by two graphitized cellulose threads connected respectively to the vibrator and the pick-up units. The vibrator was driven from a Muirhead decade oscillator and the resonance condition determined with a cathode-ray oscilloscope. The pick-up units were mounted on water-cooled supports and shielded from the furnace winding so as to keep their temperature below 50° C. The whole assembly was operated in a vacuum chamber at a pressure of 10^{-4} mm. mercury. The fundamental frequency ranged from about 800 to 2,000 c./s. for all the materials considered. The variation with temperature is relatively small, and at 1,000° C. the frequency can be as little as 50 cycles above the value at room temperature. In all cases specimens were given a preliminary degassing run to 1,000° C., under vacuum, before making measurements.

In addition to the pronounced minima, a noteworthy feature of these results is the hysteresis between heating and cooling (the direction of change of temperature is indicated in Fig. 1 by the arrows) ; this is, in general, more pronounced for the higher treatment temperatures. On re-cycling four or five times, the degree of hysteresis showed a tendency to diminish very slightly. Heating and cooling were

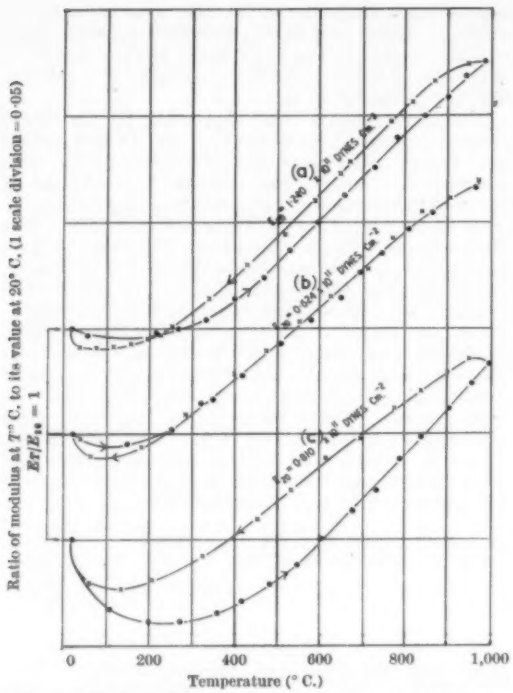

Fig. 1. Relative variation of Young's modulus as a function of temperature for Acheson reactor grade carbon (*a*) heat-treated to 1,200° C.; (*b*) heat-treated to 2,100° C.; (*c*) heat-treated to 2,600° C.

each normally carried out over a period of about 3 hr. It was observed that, if the temperature was held at some intermediate value during heating, the modulus would tend to rise toward the cooling curve. But the holding time has not yet been extended long enough (more than 19 hr.) actually to reach the 'cooling' condition. No such effect was observed on holding during cooling.

The temperature at which the minima occur is not constant but appears to lie between 100° and 300° C., depending on the nature of the material. As indicated above, the region of negative variation with temperature is of great interest, and experiments now in progress indicate that this extends down to a temperature of − 196° C. It is considered that this behaviour reflects either the behaviour of the crystal shear modulus (C_{44}) or a dilatational modulus (C_{11} or C_{33}), all of which are expected to fall with rising temperature in accordance with general theory. The occurrence of a minimum and a subsequent rise of modulus may then be attributed to some structural change which becomes important at about 200° C.

There are two possible explanations of the positive variation with temperature, and both depend on the anisotropic nature of the graphite single crystal. The first, proposed by Mrozowski[4], is that, on heating, internal cooling stresses introduced during manufacture (that is, on cooling from the final heat-treatment temperature) become relieved, with consequent increase in strength and modulus. The other, described by Hove[5], is that, on heating, the large *c*-axis expansion of the crystallites, hitherto accommodated in the voids between the crystals, enables a filling-in of these voids to take place, thus tightening up the structure; this, incidentally, allows the *c*-axis component to play an increasingly larger part in the artefact expansion as the temperature rises. At this stage it is possible to raise three objections to the first, or 'strain', hypothesis: (i) Present results indicate that any built-in strains would cease to be effective below 200° C. There is no obvious reason why this should be so. (ii) The time-dependent nature of the heating curve and the relative stability of the cooling curve suggest that annealing does go on at these temperatures, and hence that there would be little tendency for strains to build up during the much slower cooling conditions of manufacture. (iii) Greater strains (at room temperature) should be induced by the higher heat-treatment temperatures, thus resulting in steeper modulus variations. This is not in agreement with the experimental results.

Davidson and Losty[6] have objected to the second, or 'void', hypothesis. They suggest that Wigner growth of the crystal resulting from neutron irradiation ought to be related to the consequent increase of modulus of the artefact in a manner parallel to the thermal case, whereas in fact it is found that under irradiation the modulus of the artefact increases far more rapidly than in the thermal case.

However, the present facts seem to favour the void hypothesis. On elementary grounds one would expect, on the basis of either hypothesis, accompanying changes in expansion coefficient. As already pointed out, the void hypothesis requires both the modulus and the expansion coefficient to change in the same sense. On the other hand, the strain hypothesis implies that modulus and expansion should change in opposite senses. For, in this case,

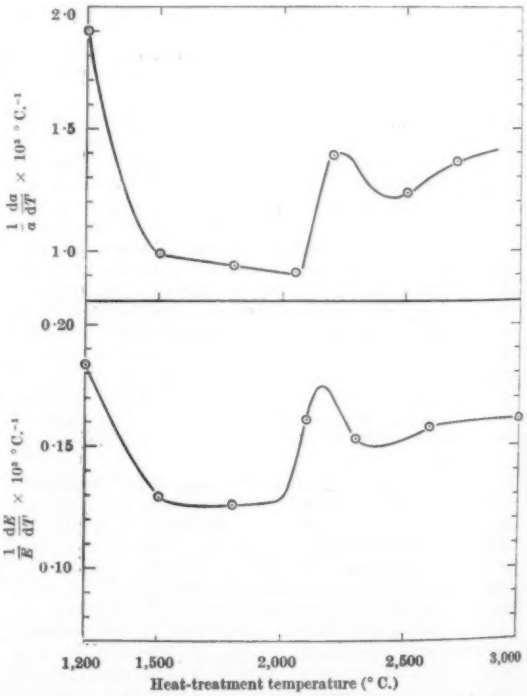

Fig. 2. Temperature variations of thermal expansion coefficient (*a*) and Young's modulus (*E*) as functions of heat-treatment temperature at ambient temperature of 600° C.

any increase in modulus must presumably arise from a net effective reduction in molecular spacing, and this would result in a fall in expansion coefficient. In Fig. 2 are shown, plotted together, the variations with temperature of modulus and expansion coefficient as functions of heat-treatment temperature. The modulus variations have been determined from the cooling curve and correspond to an ambient temperature of around 600° C. The expansion data were obtained by Mr. T. Beirne, using the push-rod-and-tube technique on $\frac{1}{2}$-in. diameter specimens in a nitrogen atmosphere. The variations have again been calculated for 600° C. ambient temperature.

There is a striking similarity in the shapes of the two curves, showing that elastic modulus and expansion coefficient follow one another very closely. It should be noted that although both modulus and expansion measurements were obtained from respective single specimens for each treatment-temperature, the two sets of specimens were cut from two distinct 8 in. × 8 in. × 32 in. blocks of carbon. The general variation with heat-treatment temperature is thus quite genuine and cannot be attributed to in-block variations in structure (although no doubt there are minor variations between specimens which would preclude a perfect agreement). This result thus gives support to the void hypothesis.

The changes in expansion are roughly ten times the changes in modulus. This is reasonable if it is assumed that the effect depends mainly on the micropores. The filling-in of these would not affect the mechanical structure very much, but it could lead to large changes in the contribution to the artefact expansion made by the c-axis component of individual crystallites.

Assuming then that void filling is the dominant mechanism, the hysteresis could arise from the inability of crystallites to expand cleanly without overcrowding their neighbours. Some general plastic yielding would then take place before the crystallites reached their full extent, and before the expansion coefficient reached the maximum for the particular temperature in question. On cooling down, the crystallites would shrink back, and this would be a relatively clean process involving rather less plastic yielding. As noted above, dependence of the cooling curve on time has not been detected.

Since the position of the minimum is always close to 200° for materials the pore structure of which is quite different, it is pertinent to ask whether this point does not represent a specific plastic yield temperature rather than some function of the pore structure. The existence of such a yield temperature would be of great interest in interpreting other properties of graphite.

Further investigations are being directed to varying the source material and to exploring thoroughly the low-temperature region.

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INFLUENCE OF PTERIDINES ON THE PRODUCTION OF VITAMIN B_{12} BY TRYPANOSOMID FLAGELLATES*

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IT was noted several years ago that a protozoan parasite of mosquitoes, *Crithidia fasciculata*, required for growth inordinately high levels of a conjugated pteridine ; folic acid or still higher levels of folinic acid fulfilled the requirement[1]. The lower efficiency of folinic acid emphasized that the role of conjugated pteridines in this organism differs from the microbial pattern in which folic acid functions only in one-carbon metabolism.

The puzzle of this strange pteridine requirement was solved when it was shown[2] that conjugated pteridines serve two functions in the metabolism of this hæmoflagellate : (1) The usual role in one-carbon metabolism ; (2) As a precursor of a new class of growth factors, the unconjugated pteridines, previously called the '*Crithidia* factor' by Nathan and Cowperthwaite[3]. We now know that the 2-amino, 4-hydroxy pteridines, which have good *Crithidia* factor activity, are those which are substituted in the 6-position with either—CH_2OH or a longer aliphatic chain with two vicinyl hydroxy groups.

So far a direct approach to the elucidation of the role of the *Crithidia* factor has been unsuccessful ; we have been unable to bypass the growth requirement for the *Crithidia* factor. However, we have recently shown that 2,4,5-triamino-6-hydroxy pyrimidine plus some sugar, such as glucose, can replace *Crithidia* factor[4]. Apparently the protozoan can use the pyrimidine as a pteridine-precursor by converting it to an activated form and closing the incomplete pyrazine ring with glucose.

Now that there are several ways available for satisfying the pteridine requirement of *C. fasciculata* (Table 1, column 1), one way to study the function of the *Crithidia* factor is to measure the variation in key products formed as a function of the compounds used to satisfy the multiple pteridine requirement. Vitamin B_{12} seemed a key product to measure because it is known that, in certain diseases involving vitamin B_{12}, folic acid sometimes relieves symptoms, whereas in other B_{12}-linked diseases, such as pernicious anæmia, folic acid potentiates neurological involvement. To check the validity of measuring the vitamin B_{12} formed by *C. fasciculata*, the B_{12}-products formed by

* Substance of a report presented at the third International Symposium on the B-Vitamins at Poznan, Poland, during September 1959.

Table 1. Effect of Variations in Pteridines supplied Exogenously on Vitamin B_{12}-activity Production by *Crithidia fasciculata*

Compound supplied/ml.	mgm. dry cells per litre	$\mu\mu$gm. B_{12}/gm. dry cells		$\mu\mu$gm. B_{12}/l. supernate		$\mu\mu$gm. B_{12}/l. (cells + supernate)	
		Euglena assay	*Ochromonas* assay	*Euglena* assay	*Ochromonas* assay	*Euglena* assay	*Ochromonas* assay
Folic acid 1·0 μgm.	220	15,000	680	700	4,500	4,000	4,654
Folic acid + 1·0 mμgm. 2-NH_2, 4-OH, 6-trihydroxypropyl pteridine 1·0 mμgm.	222	18,900	5,500	2,150	4,000	6,368	5,221
Thymidine + 20·0 μgm. 2-NH_2, 4-OH, 6-trihydroxypropyl pteridine 1·0 mμgm.	180	28,900	4,000	2,000	3,000	7,200	3,720
Folic acid + 1·0 mμgm. 2,4,5-triamino, 6-OH pyrimidine 5·0 μgm. (and glucose from the basal medium)	200	0	0	0	0	0	0

Strigomonas oncopelti, a related protozoan which requires *p*-aminobenzoic acid but no pteridine, were also assayed.

Two-litre batches of *Crithidia fasciculata* (*Anopheles* strain, *ATCC* 11745) were grown in a defined medium[5] modified by increased levels of several of the mineral components (a reflexion of the decrease in amounts of contaminating inorganic ions in the organic components of the medium and in the distilled water which is now prepared in a glass still). The organisms were allowed to incubate at about 28° C. until there were about 200 mgm. of cells (dry weight) per litre of culture. The cells were then collected by centrifugation and preserved in a lyophilized state until they were prepared for assay. The culture supernatants were twenty times concentrated and stored at 6° C. until they were prepared for assay.

Strigomonas oncopelti (*ATCC* 12982) was grown in the medium given in Table 2. Because this trypanosomid does not require hæmin as a growth factor, the *p*H-range for growth may be extended below *p*H 7·6, where hæmin would ordinarily be precipitated from the medium. For these experiments *S. oncopelti* was grown at *p*H 7·9–8·1, as was *C. fasciculata*, to eliminate the possibility of differences of product due to difference of *p*H. The same methods were used for incubation, harvesting and storage of both organisms.

250 mgm. of lyophilized cells were homogenized in 25 ml. of *p*H 4·5 aconitic acid buffer which contained 0·5 per cent metabisulphite[6]. This homogenate was autoclaved at 121° C. for 30 min., diluted to 1·0 mgm. cells/ml. with distilled water, and assayed. Culture supernatants were diluted 1 : 3 (v/v) with buffer, autoclaved, filtered through paper and assayed.

Table 2. Defined Medium for *Strigomonas oncopelti*

Potassium phosphate	0·02 per cent
Crystalline magnesium sulphate	0·08 per cent
Calcium (as chloride)	0·3 mgm. per cent
Copper (as sulphate)	0·04 mgm. per cent
Metals mix*	2·0 mgm. per cent
Sodium chloride	0·05 per cent
Triethanolamine	0·5 per cent
L-Arginine	0·03 per cent
L-Glutamic acid	0·1 per cent
L-Histidine	0·02 per cent
DL-Methionine	0·02 per cent
Vitamin mix†	1·0 ml./100
p-Aminobenzoic acid	1·0 μgm. per cent

* Metals mix: 2·0 mgm. = iron, 0·2 mgm. per cent as $Fe(NH_4)_2(SO_4)_2.6H_2O$; zinc, 0·1 mgm. per cent as $ZnSO_4.7H_2O$; manganese, 0·1 mgm. per cent as $MnSO_4.H_2O$; copper, 0·008 mgm. per cent as $CuSO_4.5H_2O$; cobalt, 0·01 mgm. per cent as $CoSO_4.7H_2O$; boron, 0·01 mgm. per cent as H_3BO_3; molybdenum, 0·005 mgm. per cent as $(NH_4)_6Mo_7O_{24}.4H_2O$; vanadium, 0·001 mgm. per cent as $NaVO_4.16H_2O$.

† Vitamins mix: 1·0 ml. = thiamine hydrochloride 0·1 mgm., nicotinic acid 0·1 mgm., calcium pantothenate 0·1 mgm., pyridoxamine dihydrochloride 0·1 mgm. per cent; biotin 1·0 μgm.

Vitamin B_{12} was measured with *Ochromonas malhamensis* and *Euglena gracilis*. We have been accustomed to assume that *O. malhamensis* measures only 'true B_{12}', that is, those forms of vitamin B_{12} utilizable by humans, and that *E. gracilis* measures 'total B_{12}', that is, those forms to which *O. malhamensis* responds plus other forms (pseudos) which are inactive for humans. Neither of these organisms responds to factor B or nucleotides in place of vitamin B_{12}. These assays could detect 1·0 $\mu\mu$gm./ml. of B_{12} activity.

As the materials used to satisfy the pteridine requirements of *C. fasciculata* were varied, the amount and kind of B_{12}-activity produced also varied (Table 1). When B_{12} was detectable there was, in the cells, more total than true B_{12}. On the other hand, for culture supernatants, the results of the assays seemed theoretically impossible: there was more *Ochromonas*-active than *Euglena*-active material (Table 1). Such a disturbing result has been noted before with human sera[7] and in our unpublished results with sera from patients with liver diseases. There may be a differing ability of the 'true', *Ochromonas*-active and 'pseudo', *Euglena*-active B_{12} to leak out of the cells, and if so, one might expect varying results for the culture supernatants freed of cells. However, one would not expect the amount of B_{12} as assayed by *Ochromonas* to exceed that given by *Euglena*. The factor responsible for greater production of *Ochromonas*-active material may either be a special form of vitamin which is only active for *Ochromonas* (and by implication for humans); or it may be some material which releases inhibition of the synthesis of B_{12} from smaller parts already present; or it may be the long-sought B_{12}-bypass factor.

The role of pteridines in the production of B_{12}-like materials is clearer. For *C. fasciculata*, when there was only enough folic acid present to satisfy the one-carbon function and all the unconjugated pteridines had to be synthesized, presumably from the 2,4,5-triamino, 6-hydroxy pyrimidine also supplied, there was no B_{12}-activity produced (Table 1). For this organism at least, (*a*) the supply of unconjugated pteridines determines whether B_{12} will be synthesized and (*b*) the amount of available conjugated pteridines, or their one-carbon metabolism products (for example, thymidine), influence the kind of B_{12} produced.

The role of pteridines in B_{12} synthesis is more strikingly demonstrated in *S. oncopelti* (Table 3). A series was arranged in which *p*-aminobenzoic acid, folic acid, or *p*-aminobenzoic acid plus various pteridines was used to satisfy the *p*-aminobenzoic

Table 3. Effect of Additions of Pteridines to the Growth Medium on the Production of Vitamin B_{12} by *Strigomonas oncopelti*

Source of *p*-aminobenzoic acid and of pteridines	(μgm./ml.)	B_{12} (μμgm.) per 225* mgm. dry cells	
		Euglena assay	*Ochromonas* assay
p-aminobenzoic acid	0·1	0	0
Folic acid	0·50	67,500	45,000
p-aminobenzoic acid + 2-NH_2, 4-OH, 6-trihydroxy propyl-pteridine	0·1 0·005	67,500	45,000
p-aminobenzoic acid + Folic acid + 2-NH_2, 4-OH, 6-trihydroxy propyl-pteridine	0·1 0·005 0·005	67,500	45,000

* ± 10 mgm. This is the cell crop per litre of medium. The supernatant fluids contained no B_{12}.

acid requirement and to supply possible stimulators for B_{12} synthesis. Production of B_{12}-activity in *S. oncopelti* cells was unusually high: 300 μμgm. *Euglena*-active material/mgm. cells as compared with 28 μμgm./mgm. cells for the best *C. fasciculata* yield and 200 μμgm. *Ochromonas*-active material/mgm. cells as compared with 5 μμgm./mgm. cells for the best *C. fasciculata* yield. Moreover, there was no effect on the balance between *Ochromonas*-active and *Euglena*-active material when the pteridines given to *S. oncopelti* were varied; it was uniformly high. In parallel with the results obtained with *C. fasciculata* (Table 1), there was no assayable B_{12} produced when no exogenous pteridine supply was introduced.

The relatively large quantities of B_{12} compounds produced by *S. oncopelti* (Table 3) suggest the use of this organism for the concentration and identification of the various forms of the vitamin produced and for the later identification of *C. fasciculata* products. The high yields and the absence of the need for exogenous hæm also point to the use of *S. oncopelti* for enzymatic studies of B_{12} synthesis.

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TURNOVERS OF ERYTHROCYTES AND PLASMA PROTEINS IN MAMMALS

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MUCH work has been carried out on the survival of erythrocytes and plasma proteins in different animals. In general, a clear pattern has emerged. Plasma proteins disappear from the circulation exponentially, as illustrated by curve *A* in Fig. 1 (refs. 1, 2). Under favourable conditions of iodination, the rates of disappearance of iodinated proteins or passively transferred antibody globulins (including protein molecules of all ages) are the same as those obtained with proteins after ^{14}C-amino-acid labelling (in which most labelled molecules are of a similar age)[1,2]. It follows that removal of plasma proteins from the circulation is a random process which is largely independent of the age of the protein molecules.

This process stands in sharp contrast to the removal of erythrocytes from the circulation. There is substantial evidence that the normal disappearance of erythrocytes from the circulation is linear rather than exponential[3] (Fig. 1*B*). Cells labelled by incorporation of isotopic glycine into hæm show survival curves of the type illustrated in Fig. 1*C*. Apart from an initial rapid breakdown, they remain at approximately constant level for a certain period, about 100 days in man, after which they rapidly disappear from the circulation[3,4]. This suggests that the factor determining their breakdown in or removal from the circulation depends primarily on some property of the aged cell, while external factors are of secondary importance. The life-span of erythrocytes seems to be controlled by an internal process.

In this article an attempt will be made to show that the turnovers of erythrocytes and plasma proteins, which at first sight seem so dissimilar, may in fact depend on comparable mechanisms. Although reticulocytes contain demonstrable ribonucleic acid[5], it is generally agreed that mature erythrocytes have no nucleoprotein[6]. Since there is substantial evidence that nucleic acid is involved in protein synthesis, it is scarcely surprising that mature erythrocytes cannot

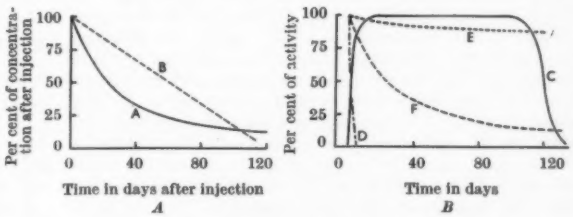

Fig. 1. *A*, concentration of labelled plasma albumin in circulation at various times after injection into human subjects; *B*, concentration of donor erythrocytes at various times after transfusion into normal human recipients; *C*, concentration of isotopically labelled hæm in circulation after a single injection of labelled glycine in human subjects; *D*, concentration of tricarboxylic acid cycle and hæm-synthesizing enzymes in human erythrocytes as a function of their age; *E*, concentrations of enzymes showing slow decrement rates (for example, lactic dehydrogenase) in human erythrocytes as a function of age; *F*, concentration of enzymes showing intermediate decrement rates and of adenosine triphosphate in human erythrocytes as a function of age

synthesize hæmoglobin or stromal protein[7]. It is a reasonable deduction that the metabolism of the non-nucleated erythrocyte, upon which the maintenance of a functional cell-membrane and hæmoglobin are known to depend, must be carried out by enzymes inherited from the immature, nucleated precursor. The suggestion has therefore been made, and supported by experimental evidence, that there is no synthesis of enzymes in mature erythrocytes and an exponential decay of enzyme activities at different rates during their life-spans[8].

In human erythrocytes, activities of hæm-synthesizing and tricarboxylic acid cycle enzymes decline very rapidly, so that there is no measurable activity in mature cells[9] (Fig. 1*D*); this decline parallels the disappearance of mitochondria from maturing cells. Certain other enzymes, for example, lactic dehydrogenase[10] and purine nucleoside phosphorylase[11], show only slight decrements in activity in old erythrocytes (Fig. 1*E*). However, several enzymes show exponential falls in activities in ageing human erythrocytes at rates intermediate between these two extremes (Fig. 1*F*); such behaviour is shown by glucose-6-phosphate dehydrogenase, phosphoglyceraldehyde dehydrogenase, DPNH·H$^+$ methæmoglobin reductase, catalase and cholinesterase[8,10,11]. It is noteworthy that the concentration of adenosine triphosphate follows a similar curve in ageing human[10] and rabbit[12] erythrocytes, suggesting that its generation is limited by decline in activity of one or more enzymes at this rate. Since adenosine triphosphate is the immediate source of energy for many important reactions in the erythrocyte, including active uptake of glucose and phosphate and active transport of ions across the cell-membrane[9], it would not be surprising if fall in concentration of adenosine triphosphate to a critical level results in the disordered metabolism and demise of the cell. It is unlikely that the hexose monophosphate shunt pathway is critical in maintaining the metabolic viability of erythrocytes under normal conditions, since subjects with considerably reduced erythrocyte glucose-6-phosphate dehydrogenase activities show normal life-spans unless exposed to primaquine or other hæmolytic agents[13].

It is of interest that curves 1*A* and 1*F* (Fig. 1) are almost superimposable, which suggests that enzymes in erythrocytes decrease in activity at rates comparable with the rates of disappearance of plasma proteins from the circulation. The decrement in enzyme activity in ageing erythrocytes is not due to diffusion out of the cells, since no activity of most of these enzymes is demonstrable in the plasma. It must be due to inactivation within the erythrocyte. Observations on erythrocyte life-spans in different species suggest that the rates of enzyme inactivation in erythrocytes, as well as rates of turnover of plasma proteins, are related to overall metabolism. It has been established and adequately documented[14–16] that small animals have higher specific metabolic rates than large animals under comparable conditions. The metabolic rates of terrestrial mammals can be expressed by the equation $O_2 = 0{\cdot}0633\ B.W.^{-0{\cdot}27}$, where O_2 is the oxygen consumption in ml. per gm. tissue per min. and *B.W.* is the body-weight in gm. (equation 1). Several fairly satisfactory methods for determining life-spans of erythrocytes have now been applied to a number of mammalian species. These methods include differential agglutination and hæmolysis after injection of cells differing in blood-group specificity from those of recipients, labelling hæmoglobin with radioactive iron and isotopic glycine, and labelling of cells with radioactive di*iso*propylfluorophosphonate. The use of radioactive chromate gives comparable results within species, but not between species, owing to differences in rates of elution from erythrocytes. Nucleated avian erythrocytes can also be labelled with phosphorus-32 in deoxyribonucleic acid. A review of these methods and their limitations has recently been published by Berlin *et al.*[3].

Representative results for different species are summarized in Table 1 (refs. 17–27). In general, there is a reasonable measure of agreement among the results for a single species, although some rather large discrepancies remain to be resolved by further work. From the data in Table 1 it is clear that the erythrocytes of small mammals have shorter life-spans than those of large mammals. When the results are presented graphically (Fig. 2), it appears that there is a straight-line relationship between erythrocyte life-span and the logarithm of body-weight. The regression of life-span (Y) in logarithm of body-weight (X) can be calculated by the least squares method and described by the equation $Y = 23{\cdot}95X - 1{\cdot}89$ (equation 2). The sheep erythrocyte has a somewhat longer life-span than expected from this relationship. However, in some experiments with sheep considerable random destruction is evident[28], so that the average erythrocyte life-span is probably less than 120 days.

From Fig. 2 and equation (1) it can be concluded that erythrocyte life-span is inversely proportional to the logarithm of metabolic rate. In keeping with such a concept is the report that in human patients with hyperthyroidism erythrocyte life-span is significantly diminished[29]; the same appears to be true from the data presented for rats made hyperthyroid[30]. Conversely, in marmots kept in the cold, erythrocyte life-spans are prolonged, the effect being very striking in hibernating animals[31]; similar observations have been made with hamsters[32]. If there are comparable activities of critical enzymes at the beginning and end of the lives of erythrocytes of different species, it can also be concluded from data summarized in Figs. 1 and 2 that the logarithmic rates of enzyme decrement are proportional to logarithms of metabolic rates.

Birds have high body temperatures and metabolic rates, and it is noteworthy that the life-spans of their nucleated erythrocytes are relatively short[22–25] despite some capacity for continued protein synthesis in the circulation. However, the life-span of nucleated erythrocytes of the cold-blooded turtle is reported to

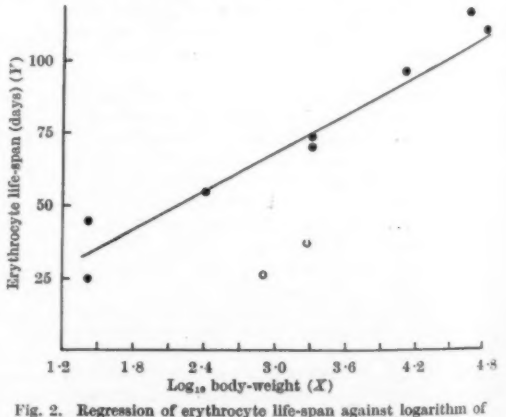

Fig. 2. Regression of erythrocyte life-span against logarithm of body-weight in various mammals (●) and two birds (○)

Table 1. Life-spans of Erythrocytes of Various Species Determined by Different Methods

Species	Body-wt. (gm.)	Potential life-span (days)	Method	Ref.
Mouse	23	20–30	Fe-59	17
Mouse	23	41–45	DFP-32	18
Rat	250	50–60	Fe-59	19
Rat	250	(55)	DFP-32	18
Rabbit	2,000	50–75 (70)	N-15 glycine ; Fe-59	3
Cat	2,000	68–77 (73)	N-15, Fe-59	20, 21
Dog	13,000	90–135 (95)	N-15, Fe-59	3
Sheep	70,000	120	Fe-59	22
Man	65,000	100–135 (113)	N-15 ; diff. agglutination DFP-32	3
Fowl	700	28–35 (31)	N-15 glycine ; P-32	23 24
Duck	1,300	39	^{14}C-glycine	25
Turtle	120	> 11 months	^{14}C-glycine	26

Values in brackets are most probable mean life-spans from available data. Body-weights, unless given, are taken from ref. 27.
DFP, radioactive di*iso*propylfluorophosphonate.

Table 2. Number of Times Erythrocytes of Various Mammals are Deoxygenated (Refs. in brackets)

Species	Blood vol. (ml.)	Cardiac output (ml./min.)	Life-span (days)	No. of times deoxygenated
Rat	20 ((33)	47 (27)	55	186, 120
Dog	1,000 (34)	1,300 (27)	95	177, 840
Man	5,500 (27)	5,100 (27)	120	160, 344

Table 3. Half-lives of Plasma Albumin in Different Animals

Group	Species	Body-wt. (gm.)	α-life (days)	Ref.
A	Cow	185,000	20·7	36
	Man	65,000	15·0	36
	Dog	13,000	8·0	36
	Rabbit	2,000	5·7	36
B	Guinea pig	730	2·8	37
	Rat	250	2·5	36
	Mouse	25	1·9	37
C	Man	65,000	23	1
	Baboon	20,000	16	38
	Rabbit	3,000	8·8	2
	Rat	250	2·6	39

be greater than 11 months[26]. The rates of incorporation of labelled glycine into circulating turtle erythrocytes were low, suggesting a low rate of replacement of cells and protein. Presumably turtle erythrocytes use up their enzymes so slowly that synthesis in the circulation can nearly maintain the *status quo*.

The relationship between decline in enzyme activity of erythrocytes and metabolic rate may be due to direct effects upon the metabolism of erythrocytes : it is reported, for example, that triiodothyronine increases phosphate turnover by erythrocytes *in vitro*[35]. But it might also be due in part to variations in cardiac output, which would affect the rates of oxygenation and deoxygenation of erythrocytes. This process, accompanied as it is by ionic fluxes across the cell membrane, is an important metabolic event for the erythrocyte. Assuming random circulation through various organs, the number of times an erythrocyte is oxygenated and deoxygenated is $C.O. \times L.S./B.V.$, where $B.V.$ is the blood volume, $C.O.$ is the cardiac output in the same units of volume per unit time, and $L.S.$ is the average life-span of the cell in the same units of time. This calculation can only be made approximately because of the difficulty of obtaining reliable and representative figures for cardiac outputs. Nevertheless it is of interest that, as shown in Table 2, the number of times an erythrocyte is oxygenated and deoxygenated appears to be of the same order in man, the dog and the rat, species differing considerably in body-weight. In view of the arguments presented here it is of interest that the rates of turnover of plasma proteins show a similar dependence on body-weights and hence on metabolic rates of mammals. The results vary somewhat, depending on the technique of labelling, and it has been considered advisable to make independent comparisons of results obtained in two different laboratories where most of the investigations have been undertaken, the Department of Pathology, University of Pittsburgh, and this Institute (groups *A*, *B* and *D*, and *C* and *E*, respectively, in Tables 3 and 4). When the logarithms of the half-lives of plasma proteins (Y) are plotted against logarithms of body-weights (X), straight lines are observed (Figs. 3 and 4). Certain considerations deserve special note, however. In the first place, the points for turnover of serum albumin in rodents evidently fall on a different regression line from those of other mammals, suggesting that the metabolism of albumin is different in rodents. This may perhaps be related to the renal glomerular filtration, re-absorption and catabolism of albumin which have been reported in rodents[41]. Secondly, it has recently been found that in albuminæmic human subjects the half-life of albumin is greatly prolonged[42], which suggests that the normal mechanism for removal of albumin is poorly developed. Further evidence that plasma protein turnovers are related to metabolism is provided by observations that half-lives of albumin

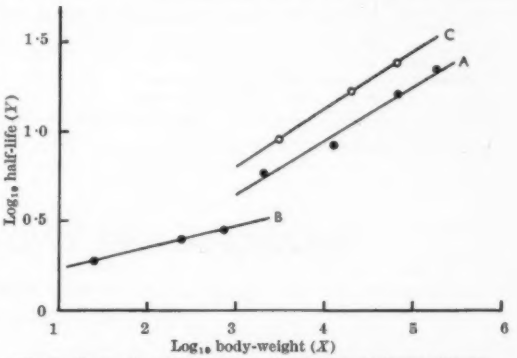

Fig. 3. Regressions of logarithms of half-lives of plasma albumin in different mammals, as shown in Table 3, against logarithms of body-weights. The values for rodents (curve *B*) fall on a different regression line from those in other mammals (curves *A* and *C*). The equations defining these regressions are : (*A*) $Y = 0{\cdot}29\,X - 0{\cdot}24$; (*B*) $Y = 0{\cdot}12\,X + 0{\cdot}12$; (*C*) $Y = 0{\cdot}31\,X - 0{\cdot}14$

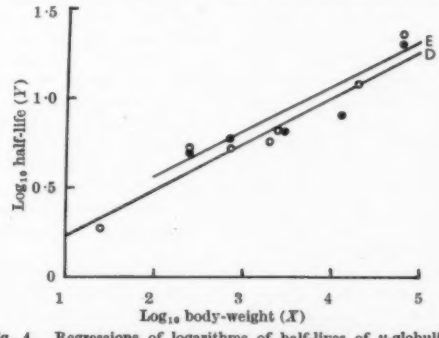

Fig. 4. Regressions of logarithms of half-lives of γ-globulins, as shown in Table 4, against logarithms of body-weights. The equations defining these regressions are : (*D*) $Y = 0{\cdot}26\,X - 0{\cdot}03$; (*E*) $0{\cdot}25\,X + 0{\cdot}06$

Table 4. Half-lives of γ-globulin in various mammals

Group	Species	Body-wt. (gm.)	Half-life (days)	Ref.
D	Man	65,000	20·3	40
	Dog	13,000	8·0	40
	Monkey	2,500	6·6	40
	Rabbit	2,000	5·7	40
	Guinea pig	730	5·4	40
	Rat	250	5·5	40
	Mouse	25	1·9	37
E	Man	65,000	23	1
	Baboon	20,000	12	38
	Rabbit	3,000	6·6	2
	Guinea pig	730	6·0	1
	Rat	250	5·4	39

and γ-globulin are both shortened by administration of thyroid hormones to experimental animals[36,40].

I am indebted to Drs. J. H. Humphrey and J. L. Fahey and Miss M. V. Mussett for advice and to Dr. A. S. McFarlane for permission to quote unpublished findings.

Note added in proof. Since this article was submitted for publication, Drs. Humphrey and Fahey have obtained evidence that the half-life of normal mouse γ-globulin is 4·6 days. Hence the regression proposed in Fig. 4 is probably not valid. The relationship of albumin half-life to body-weight shown in Fig. 3 still stands, which suggests that—as might be expected—the mechanisms of albumin and γ-globulin turnover are different.

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TRITANOPIA AND TWO-COLOUR IMAGE SYNTHESIS

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FURTHER investigations have shown the conclusions of an earlier article[1] to be essentially correct and in harmony with the interpretations of Judd[2], Walls[3,4] and Wilson and Brocklebank[3,4], but in need of clarification and the replacement of generalities with precise information. The topic originates in the fact that, under certain conditions, the image of a scene synthesized with two primary colours that approximate red and blue-green may be as acceptable to an observer anticipating a full gamut of colours as is the image conventionally rendered with three primaries[5]. Furthermore, provided additional conditions are fulfilled, the initial pair of synthesizing primaries may be chosen somewhat arbitrarily, for example, red and white (both incandescent and arc-light) or yellow and green—instead of being limited to red and blue-green[5–7].

What is distinctive in such bichromatic systems is not the red and green photographic separations used as a starting point—for these analysing primaries are common to conventional trichromatic systems as well—but the unused blue-violet separation. Thus, as with any visual system which ignores blue-violet light ($\lambda < 490$ mμ approx.), the adjective 'tritanopic'[9,16,17] applies.

There are three principal constituent phenomena at work, to each of which observers are not all equally sensitive. The first, referred to earlier[1] as "revision of the original primaries", and which will also be called the zero-order hue-change effect, is identified with 'discounting the illuminant' in the 'surface mode of perception', as proposed by Helmholtz and developed by Helson and Judd[8,9,18]. That is, on being presented with an unorthodox image, the observer's involuntary cortical processes must, in converting stimulus into perception, make a psychological choice among three possibilities: (1) a two-dimensional pattern of mere coloured patches; (2) the representation of a scene under white illumination with objects of colours the raw stimuli would evoke in isolation; or (3) the representation of a scene under tinted illumination with objects of the colours left after discounting the apparent illuminant. For example, with red and white synthesizing primaries, the choice—if not the trivial case (1)—is between (2) a white-lit scene with red, pink and grey objects, and (3) a scene with red,

white, and blue-green objects under a discounted pink illuminant.

It is convenient to employ, by way of abbreviation, the established terms[8,9,18] 'aperture' and 'surface' (or object) 'mode of perception' to indicate the choices (2) and (3), respectively. The favouring of the latter choice depends on factors of psychological significance, which may include the depiction of reality, including surface textures, or the presence of some feature seeming to be a diffuse or specular reflector of the apparent illuminant. Analogy may be drawn with another psychological problem, Necker's cube[10], where the choice is between (1) a two-dimensional pattern of twelve line segments, and (2, 3) the representation of a cube in one of two orientations with one of two corners nearest the observer. In the absence of any clues favouring one over the other, the observer's choice, in this particular case, involuntarily oscillates between (2) and (3). It is not surprising that in all such cases of cortical adaptation to a bias a choice may be made without perceptible delay; in tasks such as reading, a printed word or phrase may be recognized in an interval so short it is difficult to appreciate that a dozen or more decisions or bits of information must have been processed in that interval without conscious effort. Due to the difference and distance between stimulus and perception, a mechanical limiting of the stimulus-receiving interval, as with a 1/250-sec. shutter[12], does not necessarily prevent the processing of the stimuli during a subsequent and relatively longer blank interval, following which the perception is delivered full-grown. It is also known that no matter how short the duration of an optical stimulus, the resulting neural activity carries on for at least 1/20 sec.

In synthesized images, the chromaticity of the tinted, or biased, apparent illuminant is represented by the mixture of the synthesizing primaries, whatever their number. If the resultant is to be easily discounted to reveal a wide range of apparent underlying surface colours, it should in general be highly desaturated like the naturally occurring tinted illuminants which do not conceal too much evidence of how the underlying surface colours might appear in daylight. A light pink, for example, is especially satisfactory. Among the saturated apparent illuminants that can be simulated, only yellow (interpretable as white minus blue) is satisfactory, as it resembles the desaturated illuminants in its high intrinsic luminosity and ability to be reflected to some extent by objects of nearly all colours. In order to be discounted, a scene's apparent illuminant may be deduced from highlights or any other features which might be expected to reflect it diffusely or specularly[1], but in the absence of such clues, an average of the luminance and chromaticity integrated within the frame of the image[8,9] (in effect, a 'virtual neutral-cue') can be readily substituted[2]. Images which lack specular highlights, but are nevertheless surface-mode inducing, result when suitably crossed polarizing filters are located between photoflood lamps and camera[12], or, more simply, when the subject has a mat but textured surface, as does a woollen tapestry or carpet. Appraisal of the quality of images having nothing whatever to serve as a balance-indicating grey scale cannot, however, be intelligently undertaken, lest the seeming richness of some colours be a matter of unbalance in projector intensity rather than a genuinely enhanced effect.

Qualitatively, discounting the deduced apparent illuminant involves its subtraction—or, addition of its complementary—over the image area. To be more precise, the Judd formulæ[8] should be used. The construction of Fig. 2. of ref. 1 rapidly gives a qualitatively similar, though less precise, result. In this construction it may perhaps be logical to modify each of the original synthesizing primaries by addition of the complementary of their mixture (W) rather than the complementaries of each other. Thus S′ and L′ could lie on SW* and LW*, respectively, where W* is located between S* and L*. There is little difference in the final result beyond a foreshortening of S′L′. Another logical correction would be to replace the symbol S with the letter M throughout[2], because it is the responses of the original scene to the *l*ong and *m*iddle wave-lengths of visible light that have been recorded, while the short-wave-length response has been ignored.

In its simplest form, the zero-order hue-change effect may be demonstrated with the coloured-shadow display described by Goethe[13]. By combining on a neutral screen the light from two coloured sources, arranged so that an opaque obstacle casts two shadows, the colour of each shadow is seen to be that of one of the light sources combined with the after-image or contrast complementary of the mixture. Thus, for example, orange and yellow-green lights combine to give a very pale yellow field against which desaturated red (orange minus yellow) and desaturated bluish green (yellow-green minus yellow) shadows appear witnout perceptible delay. The shadow hues (the revised primaries) are a complementary pair; if the original primaries are capable of producing a white field by being nearly complementary and in balance at the outset, no revision takes place. That is, the question of a zero-order hue change does not arise in a two-colour image unless the synthesizing primaries are non-complementary or unbalanced at the outset. Furthermore, it will be seen later that if the synthesizing primaries (whether original or after revision) do not approximate red and blue-green, there will be little or no hue change beyond this point.

In exchanging two new for two old primaries, the zero-order effect has not in general converted a one-degree-of-freedom line of chromaticities into a two-degrees-of-freedom plane of chromaticities; the image is still bichromatic. However, certain hues, like red and blue-green, do change slightly when undergoing desaturation or when varying in luminance relative to a fixed grey scale[9,11]. For example, increased desaturation yields slight red → orange and blue-green → green shifts at increased luminance as well as slight red → purple and blue-green → blue shifts at decreased luminance. These first-order hue changes, which constitute the second constituent phenomenon and are included in the Judd-Helson formulation[8,9], can be observed in the demonstration of Fig. 1(*c*) of ref. 1. Thus, if $M'L'$ (formerly $S'L'$) roughly parallels the x axis (ref. 1, Fig. 2) the chromaticities perceived may now be chosen from a narrow ellipse of which $M'L'$ is the major axis. Other primary or revised-primary hues, such as yellow-green and violet, however, exhibit little or no such effect[11].

The zero- and first-order hue-conversion effects are essentially universal, for they occur with virtually all observers and under a variety of non-critical conditions. Much less universal is a third constituent phenomenon, to be called 'second-order hue shift,' which has been identified as an interchange between colours of equal lightness along a tritanopic confusion locus. (The effect was referred to less precisely in ref. 1 as "overlapping in the subjective distinctions between

colours of equal lightness and adjacent hue.") These particular hue shifts, which alone depart appreciably from a red : blue-green axis, require very critical photographic procedures[12], and are in general very prone to differences among individuals and dependent on subtense and context[3,4,14]. For example, in a large audience one typically finds a few young women who perceive no important hue shifts away from a red : blue-green axis, although they perceive all the lower-order hue changes readily, and are surrounded by a large body of neighbours who all see a very full gamut of hues without the slightest difficulty. For these reasons, and others given above, it is most convenient to treat the second-order effects separately, and not consider them to be lumped with the grosser lower-order effects, as Judd does[2].

In colour space, the tritanopic confusion loci are lines of constant lightness (Munsell 'value') and constant 'warmth' or 'coolness'. Typical confusion loci are those joining pale pink with warm yellow, and dark reddish brown with deep purple. The second-order shifts along these loci usually occur in the sense opposite to that of tritanopic reduction[15–17], so the term 'anti-tritanopia' may be adopted. In anti-tritanopia, a slightly greenish yellow hue component can be inserted if luminosity is high but less than that of white, while a blue-violet component can be inserted if luminosity is low but greater than that of black. Such a result might also have been predicted by the Helson effect[18] by noting that the discounted illuminant in any short-wave-deficient arrangement would be expected to be yellowish (although, in the case of red and carbon-arc-white synthesizing primaries (ref. 6, for example) the mixture is not physically yellowish at all), and that Helson specifies that a result of brightness adaptation to such an illuminant is to have relatively light patches take on the (yellowish) hue of the supposed illuminant while relatively dark patches take on the (blue-violet) hue of its complementary. However, it is difficult to identify positively anti-tritanopic shifts with the Helson effect, because the former may be so dependent on the observer, the context, or the subtense. In addition, Helson effect would not allow for the neutral sensations which are retained at all brightness levels.

The blue-violet content of natural and artificial illuminants is extremely variable and is often negligible in the latter. In addition, a part of the foveal region of the retina possesses some short-wave-absorbing pigmentation (the basic purpose of which may perhaps be to reduce chromatic aberration in a high-resolution region). Thus, the perception centres of a normal trichromat would be accustomed to the hue changes, along tritanopic confusion loci, that occur when an image moves on to the affected area of the retina, or when the illuminant varies in short-wave content, and psychological choices would frequently arise. For example, a pale pink patch could be either (1) a pink patch viewed with trichromatic organs in wide-band light or (2) a warm yellow patch viewed with short-wave-deficient organs (if of small subtense) and/or under a short-wave-deficient illuminant (if of arbitrary subtense). The involuntary favouring of choice (2) with little or no perceptible delay could be aided by factors of psychological significance, such as familiarity with the object portrayed or the fact that, in a scene, choice (1) would have led to a less complete[13] or less æsthetic picture, embodying two instead of three degrees of freedom.

Anti-tritanopia is readily demonstrated in its own right with a bichromatic set of six Munsell chips in red and blue-green hues only. The 1/2-in. mat chips 7·5 *R* 5/12 (red), 7·5 *R* 7/8 (pink), 7·5 *R* 8/4 (pale pink), 7·5 *BG* 6/6 (blue-green), 7·5 *BG* 4/5 (dark blue-green), and 7·5 *R* 3/5 (dark brown), mounted well apart and in order on a very light grey (*N* 8/0) background, are recommended. Suitable viewing conditions may be established either at arbitrary subtense with any illuminant lacking wave-lengths shorter than about 490 mμ but otherwise unselective, or at moderately reduced subtense (10′–1°, depending on the individual and the illumination-level) under white light. In either case, the sensations reported are not red, pink, pink, etc., but red, orange, yellow, green, blue and purple, respectively[3,4].

In conclusion, the perception of a multiplicity of hues in a well-executed two-colour synthesis of the image of a scene appears to be due to rapid cortical adaptation or orientation to an apparent illuminant seeming to fall on the objects in the scene. Two qualities of the apparent illuminant may be deduced : the quality of being biased away from neutrality, and the quality of being tritanopic, or of rendering only two degrees of freedom. In assessing the second quality, whether or not the first is present, what matters is not the deficiencies in the synthesizing primaries, but the supposed deficiencies in the apparent illuminant simulated by their mixture. The perceived colours are therefore formed from the original stimuli first by discounting the chromatic bias, if any, of the apparent illuminant, and then by discounting the tritanopic reduction due to its apparent omission of short-wave response and the third degree of freedom. Syntheses, the stimuli of which are not converted as described, and are therefore of great significance to some[2,7], would be merely patterns which in some way fail to be consistent with, or invite involuntary cortical interpretation as, a representation of surfaces upon which the discountable illuminants fall.

I am indebted, for exchanges of information, to Messrs. Wilson and Brocklebank (who also helped perfect the 'Tritanope's Palette'), W. B. Wintringham (who suggested that tritanopic confusion be considered), D. B. Judd, F. Pollak, and J. M. Adams. The continued interest and encouragement of R. L. Gregory and Prof. W. R. Hawthorne are gratefully acknowledged.

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LETTERS TO THE EDITORS

PHYSICS

Effect of Hydrogen-Ion Bombardment on the Oxides of Aluminium and Magnesium

COMMERCIAL high-purity, polycrystalline samples of aluminium oxide and magnesium oxide have been subjected to bombardment by a 300-μamp. beam of hydrogen ions having an energy distribution with a maximum at 500 eV. At this energy, the maximum penetration into the substances would be of the order of 100 Å., and therefore any changes of property observed would, in the main, be confined to these regions. Changes in electrical resistance have been recorded as a convenient means of following the effect of the bombardment.

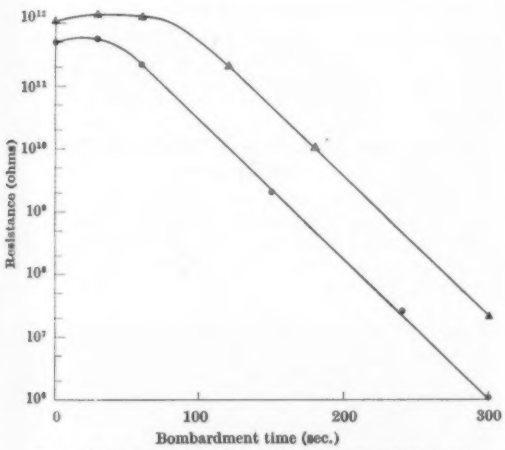

Fig. 1. Variation of resistance with bombardment time of: ○, magnesia; △, alumina

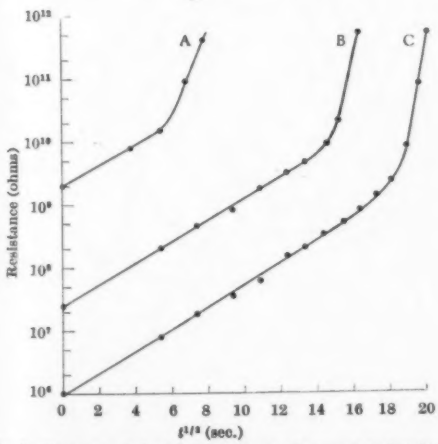

Fig. 2. Recovery of electrical resistance subsequent to bombardment, as a function of $t^{1/3}$ (sec.) for MgO. *A*, Bombarded for 150 sec.; *B*, bombarded for 240 sec.; *C*, bombarded for 300 sec.

Fig. 1 illustrates the variation of resistance with time of bombardment, and, as can be seen, there is no sign of the observed effect becoming saturated within the time-scale used. Subsequent to bombardment, it was found that the electrical resistance recovered to its original value; this is shown in Fig. 2 for the case of magnesium oxide. Aluminium oxide exhibited a similar behaviour. The full recovery process took place at room temperature, suggesting an activation energy of about 0·1 eV. In addition, the strong dependence of the recovery process on $\sqrt{t}$ (where t is time) is suggestive of a diffusion mechanism. However, further work is required before a full understanding of this effect, which may conceivably have important practical implications, is obtained.

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Submillimetre Waves by Harmonic Generation from Cold Cathode Arcs

THE use of the mercury pool arc for microwave harmonic generation has already been described[1,2]. The purpose of this communication is to report some preliminary results obtained with solid cathodes.

As before, the driving power used was a few watts obtained from a continuous-wave *Q*-band klystron operating at 35 Gc./s. The arc was mounted in a *Q*-band wave-guide and was so oriented that the ½-mm. diameter tungsten-wire anode was parallel to the microwave electric vector. The cathode material in the form of a thin slab was mounted adjustably through the *H*-plane wall of the wave-guide beneath the wire anode. The arc was struck by applying d.c. to the anode (via a variable resistance) and then moving the cathode until contact was made between it and the pointed end of the anode. The cathode was then withdrawn so that an arc less than 0·1 mm. in length was formed. Harmonic output was collected by means of a smaller wave-guide protruding through an *E*-plane wall of the *Q*-band wave-guide close to the arc. No effort was made to match the arc to the output wave-guide.

At high gas pressures (for example, 20 atmospheres argon) disintegration of the cathode material under the action of the cathode spot is much reduced; the cathode spot is clearly emitting at a very high current density, yet the cathode remains 'cold'. It seems that, in principle, any conductor may be used for harmonic generation, but for most materials the cathode spot wanders from beneath the anode wire, thus lengthening the arc and producing a startling drop in harmonic output. Calcium is the most successful cathode tried to date, but even with this material a small movement of the spot can be seen

and this produces a fluctuation in the harmonic power generated. For calcium the d.c. potential across the arc drops from about 15 volts to between 0 and 8 volts when the Q-band drive is switched on. The d.c. current (usually about 0·25 amp.) is adjusted by means of the variable resistance to give maximum harmonic output. This adjustment is very critical.

With a calcium cathode, the ninth harmonic at 315 Gc./s. ($\lambda = 0{\cdot}95$ mm.) was found, using a Golay infra-red detector for receiver. The highest output so far obtained at this frequency is about 10^{-8} watt, but if the output system can be matched to the arc, the power should approach 1 microwatt. The variation of output power with increasing harmonic number suggests that the ninth harmonic is probably not the highest present, and it is hoped to use a second arc as frequency charger for superheterodyne detection of these higher harmonics.

This communication describes work forming part of the research programme of the National Physical Laboratory, and is published by permission of the Director of the Laboratory.

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July 28.

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Dislocations in Non-metallic Layer Structures

A point not mentioned by Amelinckx and Delavignette in their communication[1] concerning dislocations revealed in thin flakes of non-metallic layer structures by transmission electron microscopy is the ready observation of grids of crossing dislocations, as distinct from hexagonal net-works, in talc and mica (Fig. 1, cf. Fig. 1 of Amelinckx and Delavignette's communication and Fig. 2).

These grids are generally remarkably stable and may be moved slightly to and fro by varying the intensity of the electron beam, although if the flake is overheated the dislocations vanish suddenly.

Fig. 1. Grid of crossing dislocations in talc (a hexagonal network is just resolvable in the bottom left-hand corner)

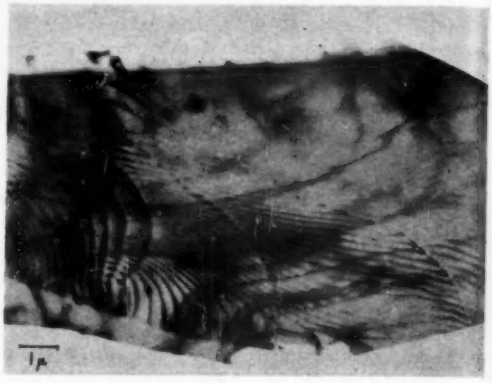

Fig. 2. Crossing of two systems of dislocations in mica

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An Electron Microscope Study of Graphite Oxidation

Lattice or surface imperfections may play an important part as active sites in the oxidation of graphite, and an attempt at observing these and how they behave on oxidation has been made by electron microscopy.

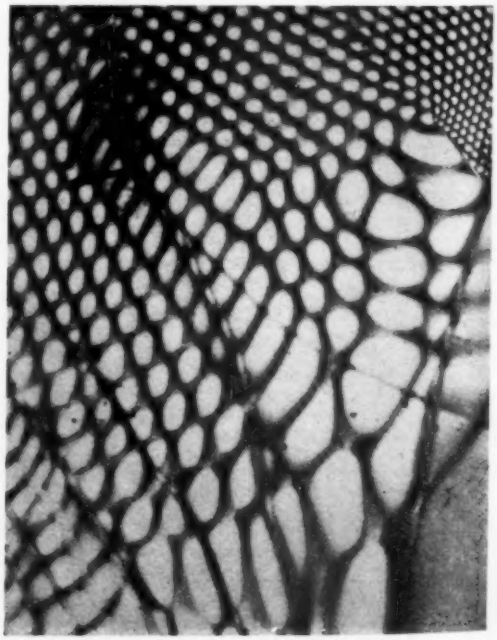

Fig. 1. (× *c.* 10,000)

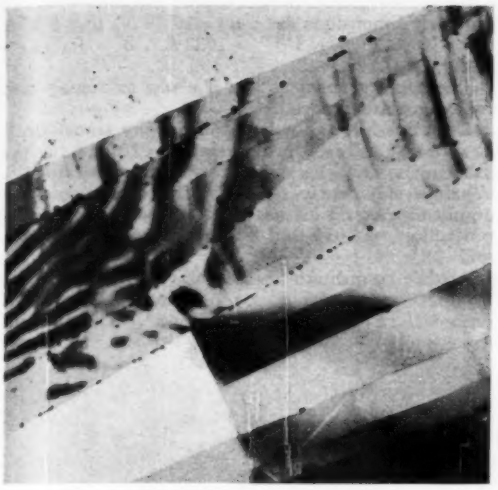

Fig. 2. (× *c.* 16,000)

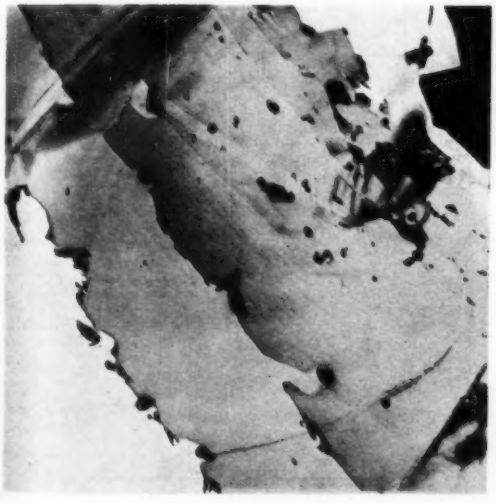

Fig. 3. (× *c.* 16,000)

Thin films of graphite were prepared by smearing microscope grids over the surface of natural Madagascar flake for examination in an *E.M.*6 Metropolitan Vickers electron microscope. These appeared as large areas of flat graphite sheets the basal planes of which were predominantly normal to the electron beam. Dislocations in the basal planes were frequently observed, the estimated density being 10^9/cm.^{2}.

Graphite is also particularly prone to the incidence of stacking faults. The basal plane dislocation splits into partials forming a ribbon of stacking fault 700–1000 Å. wide according to the equation :

$$a[10\bar{1}0] \rightarrow a/3[11\bar{2}0] + a/3[2\bar{1}\bar{1}0]$$

From measurements of the radius of curvature of dislocation nodes the stacking fault energy of graphite was estimated at approximately 0·25 erg/cm.^{2}. Interaction of partials to form networks is shown in Fig. 1.

Due to the fortuitous relative rotation of graphite sheets, moiré fringes are often observed, with dislocations imaged as extra planes. These extra lines are associated with dislocations lying in the basal planes and as these do not pass through the film the analysis of Pashley and Bassett[1] cannot be applied.

Thin films heated in air at 600° C. and examined in the microscope show that attack occurs preferentially at crystal edges and cleavage steps (Fig. 2). Further attack occurs inwards from the initial points (Fig. 3). It would be expected that greater reactivity would occur where dislocations end at crystal edges. Due to the high mobility of these dislocations, considerable movement occurs during heating and cooling and no definite conclusions can be drawn from thin films which have been oxidized outside the microscope. To overcome this, a heating stage has been designed and made for the *E.M.*6 microscope and work is now proceeding to oxidize specimens during examination.

I wish to thank the Directors of C. A. Parsons for permission to publish this communication and D. R. Ashworth for supervising the initial work.

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Ultra-violet Absorption Spectra of the Molecules H_2O, HDO and D_2O

We are in complete agreement with Barrett and Mansell[1] concerning the absorption spectrum of water down to 185 mμ, and also in their implied criticism of the work of Golding[2]. Independently of Barrett and Mansell, we had failed to observe with a Unicam *SP.*500, flushed with nitrogen, the fine-structure described by Golding, and the results were therefore checked on a vacuum grating spectrophotometer[3] and extended down to 180 mμ using silica cells of 1 cm. and 1 mm. path-length. Plotted as log *A* against wave-length, the absorbance of water rises nearly linearly down to at least 180 mμ, with no trace of fine-structure. At about 20° C. the absorbance values for 1 cm. path-length were found to be 0·1 at 192 mμ, 1·0 at 186 mμ and 10 at 181 mμ very nearly. More complete and precise results will be published elsewhere.

In the course of private correspondence by one of us with Mr. Golding, a letter from him almost identical with that which follows Barrett and Mansell's communication was received and a full reply was sent. Normal spectrophotometric precautions for work in this region were not observed if the monochromator was not flushed with nitrogen ; even in a Beckman *DK*2 spectrophotometer, the greater part of the light-path lies within the monochromator. Stray light cannot be estimated by using a shutter in the way described ; even if it could, it is of interest to compare the percentages of stray light (calculated from the figures given for maximum optical densities with less than 1 per cent error) with corresponding values determined in a more orthodox manner by Stich, Rotzler and Reichstein[4] for a specially selected *DK*2 spectrophotometer with complete nitrogen flushing (Table 1).

Table 1

	Wave-length	186 mμ	188 mμ	190 mμ	195 mμ
Percentage stray light	Golding	0·53	0·47	0·18	0·05
	Stich *et al.*	4·0	1·3	0·4	0·02

The true stray light will also not in general be appreciably dependent on width of slit; however, if any air remains in the light-path, an effect in the opposite sense to that described by Golding may be observed at the maxima of the Schumann–Runge oxygen absorption bands. Here, as the slits are made narrower, the stray light increases significantly, as might be anticipated; the sharp minima in Golding's spectra occur at the wave-lengths of these oxygen maxima, and together with the abrupt fall at still shorter wave-lengths present an excellent example of the misleading effects of stray light. The statement that Lambert's (rather than Beer's) law is obeyed must be taken with some reserve; it quite certainly would not be obeyed at the wave-lengths of the Schumann–Runge peaks. Sulphuric acid has a remarkably low absorbance down to 185 mμ, so the effect of adding water is not surprising, and of course the same is true for the effect of a solute added to one of a pair of cells containing water—in each case the increase in absorbance results in the appearance of the stray light effects.

Dr. Wilbur C. Kaye of Beckman Instruments, Inc., has informed us that with a properly flushed *DK*2 spectrophotometer the spectrum of liquid water is in agreement with the results of Barrett and Mansell, and does not show the maximum or fine structure described by Golding.

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CRYSTALLOGRAPHY

Crystal Structure of 1-3-7-9 Tetramethyluric Acid and the Molecular Packing in Hydroxymethyl Purine Crystals

The ability of 1-3-7-9 tetramethyluric acid to solubilize polycyclic aromatic hydrocarbons in water and to form 1 : 1 and 1 : 2 crystalline complexes from organic solvents, first pointed out by Weil-Malherbe[1], has recently attracted considerable interest[2,3].

Since solubilization and complex formation are closely related, we have investigated the crystal structure of some 1-3-7-9 tetramethyluric acid hydrocarbon complexes by X-ray diffraction hoping to obtain information on the interaction between the purinic and the hydrocarbon molecules. The present investigation of the crystal structure of 1-3-7-9 tetramethyluric acid was undertaken as a first step.

X-ray diffraction photographs were recorded from single crystals of 1-3-7-9 tetramethyluric acid using a Weissenberg camera and copper $K\alpha$ radiation. The unit cell dimensions are: $a = 15{\cdot}59$ Å., $b = 8{\cdot}87$ Å., $c = 8{\cdot}46$ Å., $\beta = 118°$, $V = 1033$ Å.^{3}, $Z = 4$, $d_{\text{theor.}} = 1{\cdot}430$, $d_{\text{exp.}} = 1{\cdot}422$.

The space group is $P2_1/a$ if a few extremely weak reflexions are ignored.

Both optical[4] and calculated Fourier transforms[5,6], as well as Patterson projections on 010 and 100 were used in the first stage of the structure determination. When a sufficiently large fraction of the observed amplitudes could be assigned correct signs, Fourier projections on 010 and 100 were computed (Figs. 1 and 2).

Atomic co-ordinates were refined through reiterate cycles of structure factor calculations carried out on a 650 *IBM* electronic computer[7]. The molecules

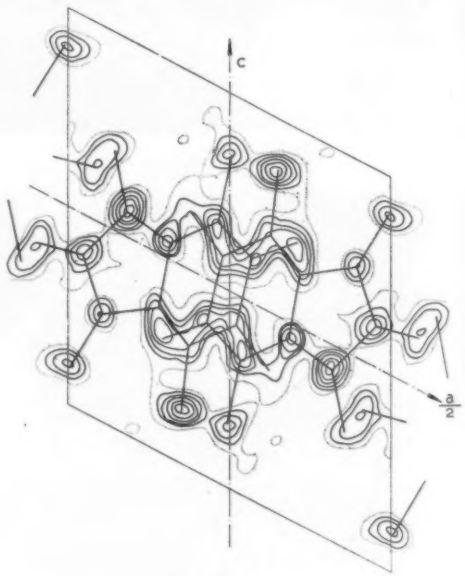


Fig. 1

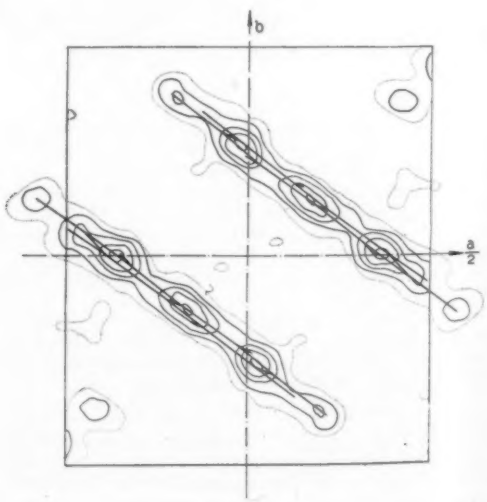


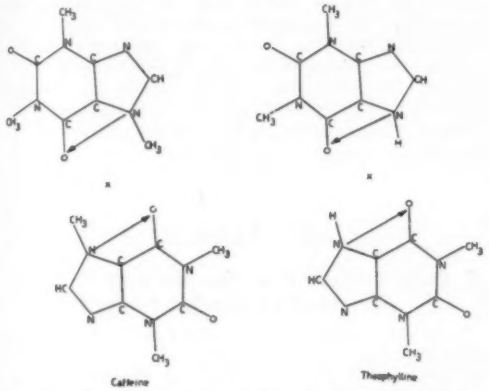
Fig. 2

appear to be planar with an average distance of the atoms from the molecular plane:

$$0{\cdot}0187\,x + 0{\cdot}4416\,y - 0{\cdot}3655\,z = 1$$

of $\pm 0{\cdot}02$ Å.

It is interesting to compare the arrangement of the molecules in the crystal with that of the two parent methylhydroxypurines, theophylline and caffeine[8]. All these structures are monoclinic, with the space-group $P2_1/a$ (ignoring weak forbidden reflexions). However, whereas the crystal structures of theophylline and caffeine are closely related, that of 1-3-7-9 tetramethyluric acid is based on a different type of packing. In caffeine and theophylline, the pairing takes place in the same plane (or almost in the same plane), according to the following scheme:



In the case of 1-3-7-9 tetramethyluric acid the two paired molecules lie in parallel planes 3·5 Å. apart, as shown in Fig. 3.

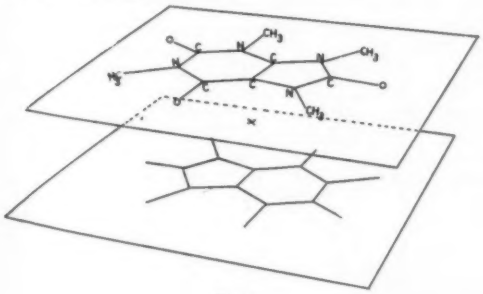


Fig. 3

From the structure of theophylline alone it might be inferred that hydrogen bonding between $\diagdown$=O and $\diagdown$N—H groups is responsible for the pairing. However, the occurrence of a similar pairing between caffeine molecules, where hydrogen bonding is ruled out by the methylation of the nitrogen, indicates that dipole–dipole interaction may be dominant in both cases. The ring nitrogen atoms forming three σ bonds probably contribute their lone pair electrons (p_z^2) to the π molecular orbitals. A large fraction of these electron pairs should be localized around the more electronegative oxygen atoms of the $\diagdown$C=O groups. The mode of pairing of theophylline and caffeine could be to the strongest $\overset{+}{\text{N}}$—$\bar{\text{O}}$ dipole, which arises from the charge-distribution in one molecule being antiparallel to the corresponding dipole in the opposite molecule, as indicated above.

In the case of theophylline, a certain degree of hydrogen bonding might be present in addition to the dipole–dipole interaction, but it would be weakened by misalignment of the oxygen lone pair orbital moments and the N—H bond moments to which hydrogen bonding is sensitive[9]. The radical change in the mode of pairing observed in going from caffeine to the 1-3-7-9 tetramethyluric acid structure can thus be explained by the weakening of the $\overset{+}{\text{N}}-\bar{\text{O}}$ dipoles resulting from the introduction of one more $\diagdown$N—CH_3 and $\diagdown$C=O group. It would appear that the strongest $\overset{+}{\text{N}}$—$\bar{\text{O}}$ dipoles are now roughly perpendicular to the long axis of the molecules, and that interaction between these dipoles is the main factor responsible for the pairing of the 1-3-7-9 tetramethyluric acid molecules in parallel planes (see Fig. 3). The role of dipole–quadrupole, quadrupole–quadrupole and higher multipole terms is difficult to estimate without calculating the π charge-distribution in the molecule.

Our results show that hydrogen bonding is not necessarily the only specific interaction responsible for the pairing of hydroxymethyl purine molecules, since dipole–dipole interaction may predominate in favourable cases.

A similar suggestion has been recently made by Cannon[10] concerning the interaction between $\diagdown$N—H and $\diagdown$C=O groups of amide bonds.

We wish to thank Dr. F. Ascoli for a gift of 1-3-7-9 tetramethyluric acid crystals. We are indebted to Prof. M. F. Perutz for valuable criticism and for reading the manuscript of this communication. This work was supported by the Consiglio Nazionale delle Ricerche and by a grant from the Rockefeller Foundation.

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As part of a programme of work on the investigation of the bond lengths and bond angles in purines[1], the crystal structure of 1,3,7,9-tetramethyluric acid has been determined. In the preceding communication, de Santis, Giglio and Liquori have reported the crystal structure of this substance, but describe a different polymorphic form from the one given here.

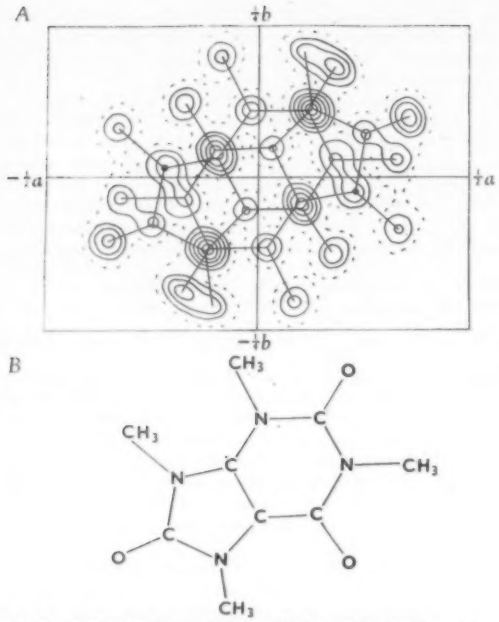

Fig. 1. *A*, *hk*0 Fourier projection with the origin at the centre. Contours are at arbitrary but equal intervals with the zero contour broken. *B*, 1,3,7,9-tetramethyluric acid showing the positions of the carbon, nitrogen and oxygen atoms in the above projection

I have found that 1,3,7,9-tetramethyluric acid crystallizes from water in two polymorphic forms. Crystals of one form are those described by de Santis *et al.*, and also by Groth when his c axis is halved. The dimensions of the unit cell given by de Santis *et al.* are :

$$a = 15{\cdot}59\ \text{Å.},\ b = 8{\cdot}87\ \text{Å.},\ c = 8{\cdot}46\ \text{Å.},$$
$$\beta = 118°,\ V = 1033\ \text{Å.}^3$$

The space group is $P2_1/a$ if some fairly weak reflexions of the type $h0l$ with h odd are ignored, but these constitute about 17 per cent of the total number of 'forbidden' reflexions. Crystals of the other form are needles elongated along the *c* axis, and the unit cell dimensions obtained from rotation and Weissenberg photographs are :

$$a = 10{\cdot}11\ \text{Å.},\ b = 14{\cdot}89\ \text{Å.},\ c = 6{\cdot}75\ \text{Å.},$$
$$\beta = 100{\cdot}4°,\ V = 999\ \text{Å.}^3$$

The space group is $P2_1/a$, there being no 'forbidden' reflexions as in the first form, and the unit cell contains four molecules. The smaller volume of this cell indicates that the packing of the molecules is more economical. These crystals appear more suitable for a full structure determination, and hence they were chosen for the present investigation.

The orientation of the molecules and their positions within the unit cell have been determined from the *hk*0 and 0*kl* sharpened Patterson functions in conjunction with structure factor graphs. The coordinates so obtained have been refined by successive Fourier syntheses, and by the method of least squares, the computations being carried out on *Mercury* in the University of London. At the present stage of refinement, the *R*-factor for the *hk*0 projection is 17 per cent and an *hk*0 Fourier projection is shown in Fig. 1.

Molecules related by a centre of symmetry lie in parallel planes approximately 3·3 Å. apart, and pairs of molecules are related by screw axes. A similar arrangement exists in the other form, the distance between parallel planes being 3·5 Å. A comparison of the two completed structures should prove interesting and should show whether there are any significant differences in the molecular dimensions brought about by the transition from one polymorphic form to the other. The occurrence of weak 'forbidden' reflexions in the other structure suggests that the polymorphic change in 1,3,7,9-tetramethyluric acid represents the rearrangement of an ordered lattice in the structure described here into a disordered one.

Full details of this structure determination and the final results will be published elsewhere.

I should like to thank Prof. E. Boyland of the Chester Beatty Research Institute for the gift of 1,3,7,9-tetramethyluric acid, and the University of London for the award of an I.C.I. Fellowship.

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METALLURGY

A Determination of the Melting Point of Element 43 (Technetium)

A small quantity (0·11 gm.) of technetium-99 metal has recently been loaned to us by Oak Ridge Laboratories (U.S. Atomic Energy Commission) and we have taken the opportunity to measure the melting point, as there does not appear to be a value reported in the literature.

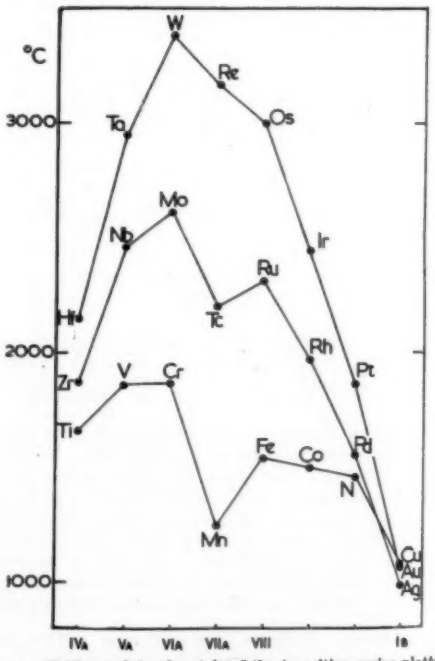

Fig. 1. Melting points of metals of the transition series plotted in order of ascending atomic number

The metal, in the form of coarse powder, was contained in a small thoria crucible, and was melted under argon in a tungsten tube resistor furnace of the type described by Haworth and Hume-Rothery[1]. The temperature was measured with a Tinsley double-scale disappearing-filament optical pyrometer, by observation through a small hole in the crucible lid. The quantity of metal was not sufficient for the direct observation of melting, but after heating to 2,150° C. the metal had not melted, while after heating to 2,250° C. the powder had melted into a small shiny bead. The melting point may therefore be taken as 2,200 ± 50° C. The molten metal did not attack the thoria, nor does it appear to be volatile at the melting point, only traces of radioactivity being detected on the radiation screens above the crucible of melt.

The melting points of metals of the three main transition series are plotted in Fig. 1 for metals of the Groups IV*A* to I*B* of the Periodic Table. It may be seen that technetium resembles manganese inasmuch as its melting point is lower than that of either of the adjacent metals in the series, the melting points of molybdenum and ruthenium, determined in the same apparatus, being 2,610 ± 20° C. and 2,310 ± 20° C. respectively.

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Crystallization of Amorphous Niobium Oxide during Anodic Oxidation

Studies of the anodic oxidation of metals such as tantalum, niobium, zirconium and titanium have been confined, in general, to temperatures of 25° C. where the thin films formed can be regarded as homogeneous dielectrics and the relationship between $V - t$, $1/C - V$ and $1/C - t$ is rectilinear.

During a study of the anodic oxidation of niobium in a variety of electrolytes (acetic, formic, phosphoric, oxalic, chloracetic, boric acids, etc., sodium chloride, strong acids and bases) it has been observed that temperature and current density have a marked influence on the anodizing characteristics. The results are illustrated in Figs. 1, 2 and 3, which show the voltage-time, weight gain-time and voltage–reciprocal capacity relationships respectively for niobium anodized in 1 *M* acetic acid at a constant current density of 1 m.amp./cm.² at various temperatures.

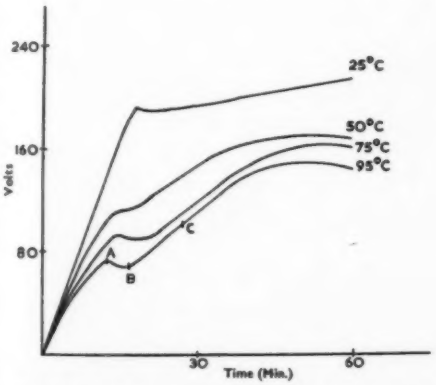


Fig. 1. Anodic oxidation of niobium in 1 *M* acetic acid at a constant current density of 1 m.amp./cm.². Voltage-time relationship at various temperatures showing plateau formation at temperatures greater than 50° C.

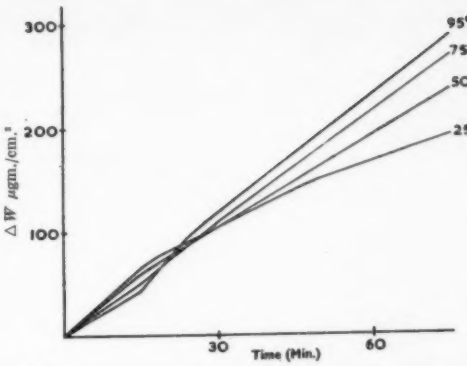


Fig. 2. Anodic oxidation of niobium in 1 *M* acetic acid at a constant current density of 1 m.amp./cm.². Weight gain (ΔW) – time relationship at various temperatures

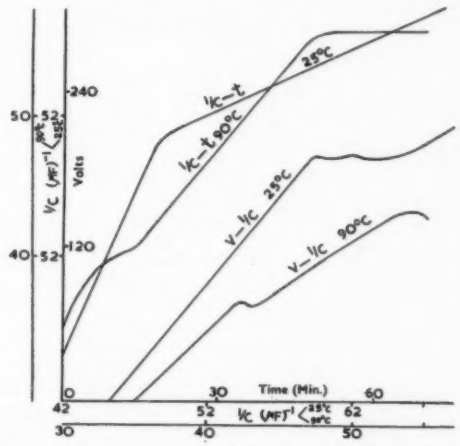
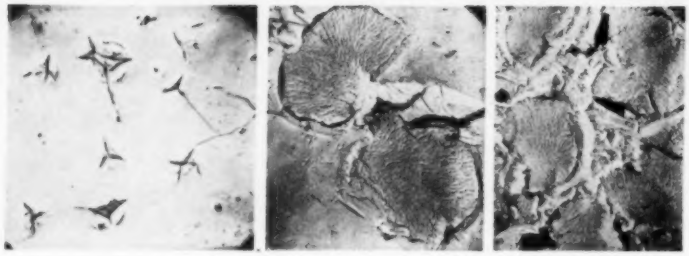


Fig. 3. Anodic oxidation of niobium in 1 *M* acetic acid at a constant current density of 1 m.amp./cm.². Reciprocal capacity–time and voltage–reciprocal capacity relationship at 25° C. and 90° C. showing corresponding changes in the slope to those in Fig. 1

At 25° C. the $V - t$ curve is rectilinear up to ~190 V. and oxidation proceeds with the formation of interference films, but at higher temperatures the $V - t$ curve is characterized by a plateau, and it is evident that the higher the temperature the lower the voltage at which the plateau occurs. Fig. 2 shows that at 90° C. there is a sudden increase in the $\Delta W - t$ curve which corresponds with the charge required for plateau formation. Similar discontinuities are evident in the $V - 1/C$ and $1/C - t$ curves (Fig. 3).

A similar plateau has been observed with the other electrolytes studied, under appropriate conditions of concentration, temperature and current density.



(a) (b) (c)

Fig. 4. Electron micrographs *a*, *b* and *c* correspond to points *A*, *B* and *C* (Fig. 1) and show the initial cracking of the film and subsequent appearance at the surface of the crystalline oxide. Magnification, *a* and *c* × 4,250, *b* × 6,500

Electron diffraction studies of films formed at various voltages at 25° C. and 90° C. have shown that at the lower temperature the oxide surface remains amorphous, but at 90° C., although the oxide gives diffuse haloes of Nb_2O_5 below the plateau, at and above the plateau the structure corresponds to crystalline Nb_2O_5.

Figs. 4*a*, *b* and *c* show electron micrographs obtained at points *A*, *B* and *C* (Fig. 1) and reveal the initiation of cracks in the surface at the plateau and the subsequent projection of crystals of Nb_2O_5 through the overlying amorphous surface layer. At 25° C., although similar cracks (similar to Fig. 4*a*) appeared at the surface at ~190 V., there was no visible evidence of crystallization even after prolonged passage of charge.

From the present work it is evident that initially the oxide surface is continuous and amorphous and that the arrest in the rise in voltage, that is, the plateau, is associated with the formation of discontinuities in the oxide which are caused by crystallization of the amorphous oxide.

Cathcart *et al.*[1] have found that during the high-temperature oxidation of niobium at 350–500° C. the growth law is initially parabolic, but, at a critical thickness which depends on temperature and time, becomes linear owing to cracking of the oxide. From marker experiments they conclude that film growth is due to anion migration through the oxide and that cracking results from the stresses which arise from the formation of the oxide at the metal/metal oxide interface.

Vermilyea[2] has described the phenomenon of field crystallization where anodically formed amorphous Ta_2O_5 has been shown to crystallize in the presence of a strong electric field and considers that rupture of the amorphous oxide is due to this crystallization which occurs within the amorphous oxide. The similarity of the photomicrographs (Fig. 4) to those obtained by Vermilyea for tantalum indicates that the phenomenon observed in the present work is due to field crystallization and that a similar mechanism is applicable.

Recent electron diffraction studies of thermal oxide films on niobium[3] have shown that the oxide is amorphous when protective but is crystalline when non-protective, indicating that film rupture is due to crystallization.

It is apparent from the present work that there are many similarities in the behaviour of niobium during anodic and thermal oxidation, as in both cases film thickening results in discontinuities in the oxide so that oxidation continues linearly. It should be observed that a protective oxide from the point of view of anodic oxidation is one where film growth proceeds to only a limiting thickness and then practically ceases owing to an alternative electrode process, that is, gas evolution. Fig. 2 shows that film growth of the anodic oxide continues owing to crystallization and consequent film rupture so that the oxide cannot be regarded as truly protective.

It is possible, therefore, that a similar mechanism occurs during the thermal oxidation of niobium and that crystallization of the amorphous oxide, rather than oxide formation at the metal/metal oxide interface as postulated by Cathcart *et al.*, is the main cause of the stresses which result in discontinuities in the oxide and a linear growth law.

Acknowledgments are made to the U.K. Atomic Energy Authority (Development and Engineering Group, Springfield) for sponsoring this research.

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RADIATION CHEMISTRY

Role of Ferrous Hydride in the Oxidation of Ferrous Ion by Hydrogen Atoms

The oxidation of ferrous ion by atomic hydrogen was recently postulated to proceed by hydride ion formation[1]:

$$Fe^{2+} + H \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} FeH^{2+} \qquad (1)$$

$$FeH^{2+} + H^+ \xrightarrow{k_2} Fe^{3+} + H_2 \qquad (2)$$

Recently the work of Dainton and Peterson[2] demonstrated the use of nitrous oxide for discrimination between various forms of hydrogen atoms (or their analogues) in γ-irradiated aqueous solutions. This technique may be applied in the establishment of the oxidation mechanism of ferrous ion by hydrogen atoms.

In the *p*H region up to 2 the radiation-chemical oxidation of ferrous ion solutions containing nitrous oxide by H atoms will proceed by (1) and (2) or alternatively by:

$$H + N_2O \xrightarrow{k_3} N_2 + OH \text{ (or } N_2OH) \qquad (3)$$

$$OH + Fe^{2+} \rightarrow Fe^{3+} + OH^- \qquad (4)$$

This scheme leads to the following results:

$$\text{(I)} \qquad G(N_2) = \frac{G_H}{1 + \dfrac{k_1\beta[Fe^{2+}]}{k_3[N_2O]}}$$

$$(II)\quad G(H_2) = G_{H_2} + \frac{G_H}{1 + \dfrac{k_2[N_2O]}{k_1\beta[Fe^{2+}]}}$$

where :

$$\beta = \frac{[H^+]}{(k_{-1}/k_2) + [H^+]}$$

and hence :

$$(III)\quad \frac{G(N_2)}{G(H_2) - G_{H_2}} = \frac{k_2[N_2O]}{k_1[Fe^{2+}]}\left(\frac{k_{-1}}{k_2}\right)\frac{1}{[H^+]} + \frac{k_3[N_2O]}{k_1[Fe^{2+}]}$$

The linear plot (Fig. 1) is obtained in good agreement with equation III. From the ratio of the slope to intercept we obtain $(k_{-1}/k_2) = 0{\cdot}05$ mole litre^{-1}. These results cannot be readily reconciled with any alternative mechanism previously proposed for this reaction :

$$(1{\cdot}1)\quad Fe^{2+}H_2O + H \rightarrow Fe^{3+}OH^- + H_2$$
$$(1{\cdot}2)\quad Fe^{2+} + H + H^+ \rightarrow Fe^{3+} + H_2$$
$$(1{\cdot}3)\quad H + H^+ \rightleftarrows H_2^+$$
$$(2{\cdot}3)\quad Fe^{2+} + H_2^+ \rightarrow Fe^{3+} + H_2$$

Mechanism (1·1) is not consistent with the dependence on *p*H of the *Gs* ratio, while according to the triple collision or the oxidation mechanism involving H_2^+ the intercept in Fig. 1 should be zero.

The oxidation of the ferrous ion by hydrogen atoms externally generated in an electrode-less discharge was recently re-investigated in our laboratory[3] over a wide region of H^+ and Fe^{3+} ions concentration. These results yield the value of k_{-1}/k_2 in the region of 0·05–0·02 mole litre^{-1}, in good agreement with the radiation-chemical results.

This ratio of rate constants is consistent with the *p*H independence of the relative rates of competing reactions of Fe^{2+} and methanol for hydrogen atoms[4]. However, the investigation of the competition between Fe^{3+} and oxygen[5] requires that k_{-1}/k_2 should be of the order of 0·01.

These results indicate the identity of hydrogen atoms produced by ionizing radiation at low *p*H and atomic hydrogen externally generated. From Fig. 1 we obtain $k_1/k_2 = 60$ and the application of the value of k_1 independently obtained[1,3] leads to $k_2 \sim 10^3$–10^4 litre mole^{-1} sec.$^{-1}$. Thus the interaction of hydrogen atoms as such with nitrous oxide is a relatively slow process, while at *p*H above 3, efficient decomposition occurs[2]. This behaviour is analogous to that of hydrogen peroxide in neutral[6] and acid[7] solutions. It appears that the hydrogen atom precursor in neutral solutions is the solvated electron, while the 'acid form' is the genuine hydrogen atom. The assumption that the acid form of atomic hydrogen involves only H_2^+ is in disagreement with the slowness of its formation[8]. This species is of importance only in acid solutions where no alternative efficient scavenging of hydrogen atoms occurs, as in the oxidation of I^- by hydrogen atoms[1,8].

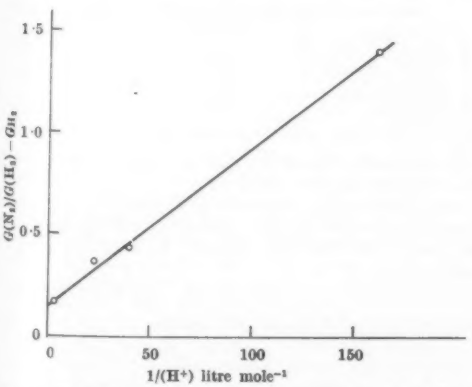

Fig. 1. Plot of the experimental results (ref. 2) for the radiation-chemical oxidation of 10^{-2} *M* ferrous sulphate solutions containing 0·01 *M* nitrous oxide

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Detection of Photochemical Oxidation of Sulphur Dioxide by Condensation Nuclei Techniques

The purpose of the tests reported in this communication was to prove that condensation nuclei techniques could be used in a qualitative way in the determination of the predominating reaction. Since quantitative performance depends on controlling several variables, proof that qualitative determinations were possible was needed to justify further work. Since sulphuric acid particles have been found to act readily as condensation nuclei, the manner of their formation from sulphur dioxide in the atmosphere was chosen as the subject of the investigation.

Several investigations have been made of the conversion of sulphur dioxide in the air to form sulphuric acid ; for example, those of Blacet[1] and of Gerhard and Johnstone[2]. They indicate that oxidation of the sulphur dioxide by ozone is insignificant compared to the action initiated by the absorption of the radiation by the sulphur dioxide molecule. The molecule then becomes activated. The series consists of the following reactions :

$$SO_2 \rightarrow SO^*_2 \qquad (1)$$
$$SO^*_2 + O_2 \rightarrow SO_4 \qquad (2)$$
$$SO_4 + O_2 \rightarrow SO_3 + O_2 \qquad (3)$$
$$SO_3 + H_2O \rightarrow H_2SO_4 \qquad (4)$$

They showed that reaction (5) is of minor importance.

$$SO_2 + O_2 \rightarrow SO_3 + O_2 \qquad (5)$$

As an example they pointed out that the addition of nitrogen dioxide, which produces ozone catalytically, did not measurably change the rate of production of sulphuric acid, whereas the rate at which sulphuric acid was produced was directly proportional to the amount of sulphur dioxide present.

The tests made here confirm their results. The production of sulphuric acid was detected by a condensation nucleus detector[3]. It has been found that the condensation nucleus detector is highly sensitive to the presence of sulphuric acid particles in

the air, a sensitivity in some cases better than one part in 10^{12}. However, because several variables affect performance, it was only possible to establish a threshold sensitivity. Some of these variables are the length of time the gas is irradiated by the light, the amount of time that the nucleogenic molecules have to coagulate, and the presence of water vapour to aid in the coagulation process.

Two different arrangements of the apparatus were made to determine what process might predominate. They were designed so that if ozone were the predominant factor in the conversion, the first arrangement would be the more sensitive, whereas if sulphur dioxide irradiation was the predominant factor, the second arrangement would be the more sensitive. In the first arrangement the air was irradiated by the ultra-violet while the sulphur dioxide that was mixed with it was not irradiated. When this procedure was followed no condensation nuclei were produced for several minutes. Those occurring at this time are believed to be due to backward diffusion from the reactor volume of sulphur dioxide into the irradiator, due in part to the pulsating flow of the condensation nuclei detector.

In the second arrangement, the irradiation tube was thoroughly purged with sulphur dioxide so that no air would be irradiated. With the light off, the mixture of sulphur dioxide and air did not produce condensation nuclei. When the irradiator was turned on, the condensation nuclei meter became saturated as soon as the irradiated sulphur dioxide had a chance to pass through the reactor volume to the detector. Due to the extreme sensitivity, it was impossible to introduce such a small amount of irradiated gas that the detector did not become saturated.

Indications are that the life-time of the irradiated sulphur dioxide is quite long. Saturated response was still obtained 30 hr. after irradiation. Evidence that the irradiated sulphur dioxide gas might have been adsorbed by the glass tubing was obtained. It was necessary to purge the tubing for several hours before there was no indication of release of irradiated gas into the condensation nuclei detector. Back diffusion of the irradiated gas into the lecture bottle used as a source of gas necessitated a change to a fresh bottle after a few experiments had been performed.

The irradiator consisted of a special mercury vapour lamp in a quartz tube parallel to another quartz tube through which the air or gas sample could pass. This allows the ozone-producing 1849 Å. radiation to reach the air in the sample tube, as well as passing those lines responsible for absorption in sulphur dioxide.

This experiment shows that it is possible to use the condensation nucleus technique in making analytical studies of reactions in the atmosphere. It has corroborated results made by more elaborate analysis.

I am grateful to Mrs. J. H. Winn for technical assistance.

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Rapid Analysis of Erbium by Neutron Activation followed by γ-Ray Spectrometry

It is known that when natural erbium is irradiated with neutrons, erbium-167*m* (2·5 sec.) is formed[1].

In the present work, a sample (10^{-3} gm.–1 gm., sealed in a polyethylene capsule) is placed in the nuclear reactor *JRR*–1 for 3 sec. to be irradiated with neutrons at a flux of 4×10^{10} neutrons per cm.^{2}/sec. (when operated at 5-kW. level), cooled for 5 sec., counted for 3 sec., again cooled for 2 sec. and counted for 3 sec. This is done by manual operation. The counting apparatus used is a 256-channel pulse height analyser attached to a 2-in. thick sodium iodide crystal.

In the resultant gamma spectrum the height of photopeak due to 0·208-MeV. gamma-ray of erbium-167*m* is measured.

The relation between photopeak height and the amount of erbium has been obtained by use of standards which involve erbium in various amounts ranging from 0·01 to 1 mgm.

The coefficient of variation of single analyses has been obtained by use of seven standard samples, each of which includes 156 μgm. of erbium. The coefficient estimated from seven values is 9·9 per cent, when the average photopeak height is 116 counts per channel and the average area of the peak is $1·9 \times 10^3$ counts. Most of the variation seems to originate from error in time measurement, especially for the beginning of irradiation.

Results of analyses are shown in Tables 1 and 2.

Table 1. Results of Analyses for Synthetic Samples* made of Rare Earths

Sample	Amount of rare earths except erbium (mgm.)	Erbium added (μgm.)	Erbium found (μgm.)	Recovery (per cent)
A	0·39	94	94	101
B	1·95	156	164 124	105 80
C†	1·95	44	28	64
D	1·95	18·7	19·5	104
E	1·95	8·7	9·0	103

* Rare earth elements, except erbium, were mixed in the ratio of natural mixtures (ref. 1).
† The ratio of erbium to other rare earths is equal to that in the natural mixtures.

Table 2. Results of Analyses for Samples to which no Erbium was added

Sample	Amount of sample (mgm.)	Erbium found (p.p.m.)
A (Monazite, Malay)	4·57	$3·5 \times 10^3$
B (Orthite, Korea)	42·4	$< 9 \times 10^1$
C (Soil, dried)	290	$< 3·8 \times 10^1$
D (Vegetable, dried)	1,291	$< 4·0 \times 10^0$
E (Holmium oxide)	14·2	$< 3·0 \times 10^2$

It is hoped to publish a full account of this work in the *Nippon Kagaku Zasshi*.

I am indebted to Prof. Shoji Makishima for helpful suggestion, to Dr. Toshio Nakai for use of laboratory facilities, and to Mr. Muneo Handa for helpful assistance.

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CHEMISTRY

Formation of Vibrationally Excited Carbon Monosulphide and Sulphur by the Flash Photolysis of Carbon Disulphide

It has recently been shown[1] that a measurable over-population of the excited vibrational levels of a diatomic molecule can be achieved by subjecting it to a high-intensity flash of ultra-violet light in the presence of a high pressure of inert gas, which maintains isothermal conditions. With nitric oxide, electronic excitation :

$$NO.X^2\Pi + h\nu \rightarrow NO.A^2\Sigma^+$$

was followed either by fluorescence or quenching, and by both mechanisms, molecules were produced in the ground electronic states with vibrational energy in excess of the zero point in high quantum yield. The excitation was made to occur without chemical reaction. These phenomena will be of considerable value in the study of energy transfer.

We describe here an extension of the same principle and method to the vibrational excitation of a transient species, produced by photochemical dissociation of a stable molecule. Electronic excitation of the transient by secondary absorption of light ultimately results in the formation of vibrationally excited ground electronic state molecules.

We have found that the carbon monosulphide molecule, produced by flash photolysis of carbon disulphide, is a suitable molecule for a preliminary search for this effect, since it is long lived and has a high absorption coefficient. The main system of carbon monosulphide ($A^1\Pi - X^1\Sigma^+$) (refs. 2, 3, 4) was first observed in absorption by Kondratiev[5] and later by Porter[6], who showed that the $C^1\Sigma - B^1\Sigma$ system also occurs in absorption and thereby identified the $B^1\Sigma$ state with the $X^1\Sigma^+$ ground-state. Our re-investigation of the flash photolysis of carbon disulphide, using a medium quartz spectrograph (Hilger *E* 498), has revealed four important new features.

(1) The carbon monosulphide molecules can be observed in the ground-state with up to at least four vibrational quanta, and the simultaneous cascading of these vibrationally excited molecules down to the level $v'' = 0$ can be followed by kinetic spectroscopy. An example of this is illustrated by Fig. 1, where the charge composition was 0·5 mm. carbon disulphide with 750 mm. of argon, 2,000 joules energy ; bands of carbon monosulphide with v'' up to 3 are marked below the diagram. Similar results were obtained using nitrogen as inert gas. The larger the value of v'', the faster is the rate of decay. It was demonstrated that the vibrational energy was out of thermal equilibrium, by carrying out similar experiments using various pressures of carbon dioxide as inert gas, in which case the absorption spectra of carbon monosulphide molecules with $v'' > 0$ were not observed. If the production of vibrationally excited molecules had been due to an increase of temperature, the effect would have been similar to any inert gas, provided the heat capacity of the system was kept constant. The vibrational energy is probably transferred rapidly to carbon dioxide if it is used as inert gas. Similar proof that the vibration energy is out of equilibrium was obtained by including butene-1 in the carbon disulphide/argon mixtures, which at quite low partial pressures (1 mm.) reduced markedly the concentration of $CS.X^1\Sigma^+(v > 0)$, and greatly increased their rate of decay. The *Q* heads of the main system of carbon monosulphide are suitable for quantitative measurement by plate photometry, and the change with time of the vibrational distribution is being investigated. Preliminary measurements show that the rate of production of $CS.X^1\Sigma^+(v > 0)$ depends roughly on the square of the flash energy, and it may be concluded that the mechanism of vibrational excitation is similar to that referred to above for nitric oxide. The concentration of carbon monosulphide is proportional to the flash energy and the rate of vibrational excitation by secondary absorption of light is proportional to the flash energy, to give an overall square dependence.

Also shown in Fig. 1 are the formation and decay of part of the $v'' = 0$ progression of sulphur. In experiments where even the smallest trace of oxygen was present, the spectrum assigned by Jones[7] to sulphur monoxide (S_2O_2) in the wave-length region 2700 Å.–3300 Å. was observed very strongly in absorption.

(2) The sulphur vapour spectrum ($B^3\Sigma_u^- \leftarrow X^3\Sigma_g^-$) in the region 2900 Å.–3700 Å., arising from reactions of sulphur atoms produced in the primary step, showed that a fraction of these molecules are also produced with excess vibrational energy ; transitions were observed corresponding to v'' up to at least 7. Polymerization of the sulphur occurred rapidly, causing a fast decay of molecules with all v'' values. The fast decay of sulphur relative to carbon monosulphide was reported by Porter[6].

(3) In addition to the two known systems of carbon monosulphide, some new bands, probably of carbon monosulphide, were observed in absorption, an apparently single-headed band being particularly strong at 2589·04 Å. (air), shaded to the red, which may occur from a transition from the zeroth vibra-

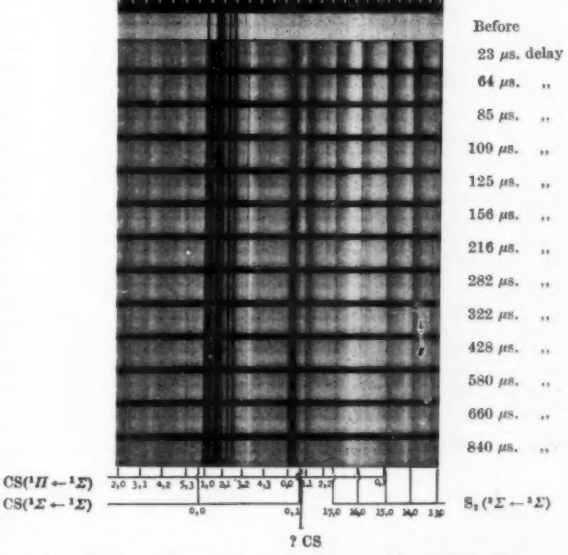

Fig. 1. Formation and decay of vibrationally excited carbon monosulphide by the flash photolysis of carbon disulphide. 0·5 mm. carbon disulphide with 750 mm. argon. 2,000 joules

tional level of the $X^1\Sigma^+$ state. (An alternative explanation is that the new bands are due to an impurity which is formed with, and decays with, carbon monosulphide. However, we were unable to relate them to known spectra which might arise from contamination of carbon disulphide.)

(4) With low pressures of carbon disulphide, < 0·05 mm., the fluorescent spectrum of carbon monosulphide was excited with such intensity by the photolytic flash that the $v' = 0$, 1 and 2 progressions could be recorded on Ilford *HP*3 plate from one exposure with a slit width of 0·01 mm. The band at 2589·04 Å. referred to under (3) occurred only very weakly in emission. At the higher pressures of carbon disulphide that were required to produce a measurable over-population of the vibrational levels of carbon monosulphide, the fluorescence was completely quenched.

Thus we summarize the mechanism of vibrational excitation of carbon monosulphide as follows:

$$CS_2 + h\nu \rightarrow CS(?) + S(?)$$
$$CS(?) \rightarrow CS.X^1\Sigma^+(v = 0)$$
$$CS.X^1\Sigma^+(v = 0) + h\nu \rightarrow CS.A^1\Pi\ (v = 0 \text{ and } 1)$$
$$CS.A^1\Pi\ (v = 0 \text{ and } 1) + M \rightarrow \rightarrow CS.X^1\Sigma^+(v = n) + M$$

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Influence of Moisture on the Spontaneous Heating of Coal

Research into the spontaneous heating of coal has been concerned in the past with three well-defined problems. These are measurements of rates of oxidation of coal, measurements of heat produced by oxidation and thirdly the establishment and solution of differential equations describing rises in temperature in oxidizing coal. Recently, measurements of the heat of oxidation of coal in air of controlled humidity have been reported and also heats of desorption and adsorption of water vapour[1,2].

In these experiments, sub-bituminous coals (Table 1) always showed a greater rate of loss of heat by evaporation of moisture in a dry air stream than their rate of heat generation by oxidation. This effect is illustrated in Fig. 1, which compares heat produced by oxidation of 4 gm. of coal with loss of heat by drying the same coal in a stream of dry nitrogen of 10 ml./min.

As a result of these experiments it has recently been pointed out that transfer of heat by evaporation of moisture can be of over-riding importance in spontaneous heatings although this factor has previously been neglected[3]. For example, the linear transfer of

Table 1. Typical Analysis of Sub-bituminous Coal, Ohai, Southland

As received basis (per cent)		By ash-free and sulphur-free basis (per cent)	
Moisture	17	Carbon	75·0
Ash	5	Hydrogen	5·4
Volatile matter	37	Nitrogen	1·1
Fixed carbon	41	Oxygen	18·5

Gross calorific value, 10,000 B.T.U.
Sulphur 0·5 per cent

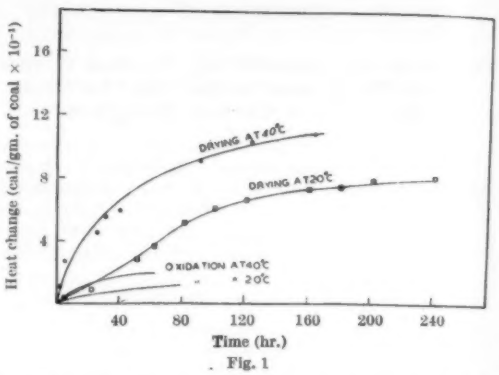

Fig. 1

heat by conduction across a temperature gradient in crushed coal is about 3×10^{-4} cal./sec./cm.[2]. If saturated air travels across this gradient and remains saturated but rises in temperature by 1 deg. C., then the increase in heat content of the air is 1×10^{-3} cal./ml. of air and a flow as low as 0·03 ml./sec. across 1 sq. cm. can be responsible for transfer of heat equal to that caused by conduction. Observations of spontaneous heating of coal heaps in New Zealand mines confirm that air travelling through the heaps does remain saturated, though as yet rates of air flow have not been measured.

These results have led to an investigation of the behaviour of sub-bituminous coal the moisture of which has been removed in a vacuum of 0·05 mm. mercury, at 105° C. The coal was crushed to the sizes shown in Table 2 and evacuated in less than 5 min. to minimize oxidation.

Table 2. Size Analysis of Coal used in Spontaneous Heating Laboratory Experiments

+ 1/10 in.	B.S.S.	19 per cent
− 1/10 in. + 60 mesh	B.S.S.	68 per cent
− 60 mesh	B.S.S.	13 per cent
		100 per cent

It has been found that 3 kgm. of coal treated in this way will fire spontaneously in 30 min. from a temperature of 22° C. when oxygen saturated with moisture at this temperature is passed through it at 1 litre/min. The coal is contained in a light aluminium foil cylinder, 12 cm. in diameter and 24 cm. high, with an open top. Air or oxygen enters the coal at a central point 2 cm. from the bottom of the cylinder through a 3-mm. diameter glass tube pushed through the coal from its top surface. Saturated air at 1 litre/min. caused ignition in 12 hr. while dry oxygen ignited the coal in 12 hr. from a temperature of 18° C.

Besides providing a striking demonstration of the importance of humidity in spontaneous heating, this experimental technique allows the spontaneous heating of coals to be studied in the laboratory under conditions of heat transfer resembling those found in practice.

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Fractionation of Polystyrene by Gel Filtration

In a recent paper[1], Porath and Flodin have described a simple and rapid method for the fractionation of water-soluble substances. In this method, which they call gel filtration, aqueous solutions of the substances are applied to the top of a column containing swollen insoluble grains of cross-linked dextrans and are eluted with water; fractions are collected from the bottom of the column in order of decreasing molecular weight. Porath and Flodin ascribe this phenomenon to a form of molecular sieving, the efficiency of which depends on the mesh size of the swollen cross-linked beads. For example, small molecules which penetrate the gel particles will be retarded, while large molecules, which are completely excluded from the gel phase, will migrate without retention in the interstitial fluid.

This communication reports some preliminary experiments in which this technique has been applied to a non-aqueous system, the fractionation of a sample of polystyrene in benzene solution.

Since polystyrene is soluble in non-aqueous solvents such as benzene it is necessary to use substances which swell in these solvents for packing the column. Insoluble cross-linked polystyrene (*PSX*) beads were used, since it is comparatively easy to prepare such beads with different degrees of swelling (for example, by using various concentrations of the cross-linking agent, divinyl benzene) and thus gels with different mesh sizes. The beads were prepared by suspension polymerization[2] and the small quantities of soluble polymer, which these beads contain, were removed by packing a column with swollen beads and eluting with benzene until no further polymer could be detected in the eluant.

A sample of polystyrene, having a wide distribution of molecular weights[3], was fractionated by a typical chromatographic elution technique using *PSX* beads of different degrees of swelling (measured by observing the swollen volume of a known weight of beads in the presence of excess benzene). The fractions were recovered by freeze-drying the benzene solutions at the water pump followed by drying to constant weight at 60° C. and were characterized by measuring the specific viscosity ($\eta_{sp.}$) at one concentration (usually 0·4 gm./100 ml.) in toluene at 30° C.

In Fig. 1 the values of $\eta_{sp.}/c.$ are plotted against the cumulative weight together with two reference standards: (*a*) the horizontal line, which is $\eta_{sp.}/c.$ for the whole polymer and represents no fractionation; (*b*) a plot of the results obtained from a conventional fractionation using the solvent volatilization method[3].

It will be seen that whereas *PSX* beads which swell only 2·2 times give practically no fractionation, with beads having higher degrees of swelling fractionation appears in the lower molecular weight region. In the case of those that swell 51 times, the resolution in this region approaches that of the solvent volatilization method. However, the degree of fractionation in the high molecular weight region is much less pronounced, indicating that even with these beads the degree of cross-linking is not loose enough to allow the larger molecules to penetrate the network.

These preliminary experiments have established, therefore, that high polymeric materials, such as polystyrene, can be fractionated by this technique, and it is thought that, by preparing *PSX* beads

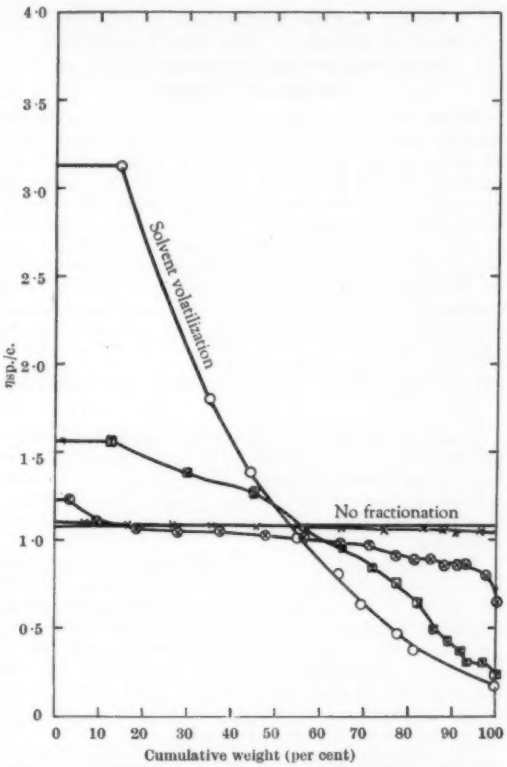

Fig. 1. Fractionation with cross-linked polystyrene beads. ×, *PSX* 524, degree of swelling 2·2; ⊗, *PSX* 509, degree of swelling 13; □, *PSX/S* 2, degree of swelling 51

having a higher degree of swelling and/or by modifying the column technique, the efficiency can be improved to approach that of the conventional fractionation methods. The possibility arises, therefore, of developing a simple method of polymer fractionation in which the fractions can be collected automatically and which is economical in the use of solvents, since most of the benzene can be recovered during the freeze-drying.

A further possible development could be in the separation of very low molecular weight materials (cf. Porath and Flodin, who cite the separation of glucose from dextran fractions of $\overline{M}_n$ 1,000 and 20,000), since although practically no fractionation took place with the *PSX* beads which swell 2·2 times, a little did, in fact, occur, suggesting that the smallest polystyrene molecules enter the *PSX* beads.

I wish to thank Miss S. A. Monksfield and Miss H. M. Sankey for assistance with the experimental work and Mr. D. Reichenberg for supplying the *PSX* beads 509 and 524, and also for his advice on the column procedure. This work is published by permission of the Director of the National Chemical Laboratory.

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Nitric Oxide Reduction in Methyl Nitrite Pyrolysis and the Transition from Isothermal Decomposition to Adiabatic Flame

When the lower alkyl nitrite and nitrate esters[1,2] (*R*ONO and *R*ONO$_2$) decompose isothermally, they do so by initial fission of the O–N bond yielding an alkoxyl radical *R*O· and the appropriate oxide of nitrogen. This step commonly controls the rate of isothermal decomposition; the subsequent reactions of the initial products[2] determine the stoichiometry. It has generally been assumed that the greater extent of decomposition of nitrates reflects the superior reactivity of nitrogen dioxide, while in the nitrites[1] isothermal decomposition scarcely involves nitric oxide at all:

$$CH_3ONO(g) \rightarrow NO(g) + 0{\cdot}5\ CH_3OH(g) + 0{\cdot}5\ CH_2O(g)$$
$$\Delta H \text{ c. zero} \qquad (1)$$

Views on the inertness of nitric oxide in the methyl nitrite system, first challenged by Carter and Travers[3], had to be further revised when the methyl nitrite self-decomposition flame was discovered[4] in 1955; this flame depends on nitric oxide reduction for its existence.

Two questions arise: (1) How are the adiabatic flame and the isothermal decomposition related?, and (2) How is the transition from decomposition to exothermic flame accomplished?, for if normal decomposition does not proceed beyond nitric oxide and is genuinely thermoneutral, this transition can be induced only by external agencies. The investigation of these questions has thrown new light on the true course of isothermal decomposition.

The adiabatic flame. It has been found that the overall stoichiometry of the decomposition flame lies between the extremes predicted[4]. The products are basically the same as those reported earlier[5,6] and reaction may be written:

$$CH_3ONO \rightarrow 0{\cdot}72\ CO + 0{\cdot}56\ H_2 + 0{\cdot}54\ NO + 0{\cdot}42\ H_2O + 0{\cdot}14\ N_2 + 0{\cdot}13\ CH_3OH + 0{\cdot}10\ CH_2O + 0{\cdot}06\ NH_3 + 0{\cdot}05\ N_2O + 0{\cdot}02\ 'CH_2NOH' + 0{\cdot}02\ CH_4 + 0{\cdot}01\ CO_2 \qquad (2)$$

The overall change in enthalpy thus resulting in the flame is $-27{\cdot}0$ kcal.mole^{-1} and this corresponds to a theoretical adiabatic flame temperature of 1,110° C., which agrees very closely with the experimental[5,7] value of 1,100° C.

Thermal decomposition and flame. Thermal decomposition of the pure vapour at 1 atm. was first studied in a flow system to simulate conditions of the stationary methyl nitrite flame. Even at 250° C., nitric oxide is reduced and water is formed. Reaction is correspondingly exothermic and thermocouple measurements show a 50 deg. rise in temperature. Thus a path is open by which methyl nitrite pyrolysis may become self-supporting; its potentialities are enhanced by the flexibility of a complex reaction mechanism; as the temperature rises so the stoichiometry alters and more heat is released. Equation 3 adequately represents the decomposition products up to about 500° C. where the self-heating is even more marked.

$$CH_3ONO \rightarrow 0{\cdot}76\ NO + 0{\cdot}46\ CH_2O + 0{\cdot}24\ CH_3OH + 0{\cdot}19\ H_2O + 0{\cdot}12\ N_2O + \text{some polymeric product}$$
$$\Delta H = c.\ -7 \text{ kcal.mole}^{-1}. \qquad (3)$$

In the neighbourhood of 590° C. the change in stoichiometry is particularly rapid, and thereafter differs very little from that of the flame. By 700° C., a degree of self-heating of nearly 300° C. is achieved; equation 4 summarizes the course of reaction:

$$CH_3ONO \rightarrow 0{\cdot}536\ NO + 0{\cdot}10\ CH_2O + 0{\cdot}11\ CH_3OH + 0{\cdot}42\ H_2O + 0{\cdot}75\ CO + 0{\cdot}04\ N_2O + 0{\cdot}151\ N_2 + 0{\cdot}06\ NH_3 + 0{\cdot}01\ CO_2 + 0{\cdot}01\ CH_4 + 0{\cdot}02\ 'CH_2NOH'$$
$$\Delta H = c.\ -26 \text{ kcal.mole}^{-1}. \qquad (4)$$

Isothermal decomposition. To establish whether the nitric oxide reduction discovered previously is merely a consequence of self-heating, the low-temperature pyrolysis of methyl nitrite has been examined under conditions of constant temperature.

Decompositions were carried out in aged 'Pyrex' reaction vessels using static systems (178–310° C.) and flow systems (235–315° C.). Velocity constants and the mean activation energy (36,400 cal.mole^{-1}) agree with old measurements[1]. Nitrous oxide is always formed, the yield never dropping far from 0·13 mole N_2O per mole nitrite decomposed. This figure is independent of temperature (from 235 to 315°), of reactant partial pressure (from 10 to 60 mm. mercury), and of percentage decomposition (from 7·7 to 100). Thus, nitrous oxide is a normal product of isothermal methyl nitrite decomposition:

$$CH_3ONO \rightarrow 0{\cdot}74\ NO + 0{\cdot}13\ N_2O + 0{\cdot}66\ CH_2O + 0{\cdot}30\ CH_3OH + 0{\cdot}12\ H_2O \qquad (5)$$

The mechanism of nitrous oxide formation proposed by Levy[8] in work on ethyl nitrite seems attractive here. Nitric oxide abstracts hydrogen from free methoxyl and the nitroxyl formed is the source of nitrous oxide. Added nitric oxide (molecular ratio NO : CH_3ONO is 12 : 1) doubles the nitrous oxide yield without sensibly affecting the rate of reaction:

$$CH_3ONO \rightarrow CH_3O\cdot + NO;$$
$$CH_3O + NO \rightarrow CH_2O + HNO;$$
$$2\ HNO \rightarrow H_2O + N_2O$$

At low temperatures, nitrogen is never found. It arises only at elevated temperatures, where it may be a product of secondary reactions undergone by nitrous oxide.

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Esterification of Fatty Acids with Glycerol

Since Berthelot's classical work on the esterification of fatty acids with glycerol, in the last century, considerable work has been done on the topic[1–4]. The presence of 2-monoglyceride in technical products of glycerolysis has been reported recently[5]; this was not noticed by Feuge *et al.*[4] or by Becker *et al.*[6] on the ground that 2-monoglyceride is prone to be quickly isomerized to the more stable 1-monoglyceride at the high temperature of the reaction. The present

Table 1. ESTERIFICATION OF FATTY ACIDS BY GLYCEROL

Temperature, 180 ± 1° C., 0·1 per cent catalyst; relation in nitrogen atmosphere

Time (min.)	Lauric		Myristic		Palmitic		Stearic		Oleic		Linoleic	
	1-MG*	2-MG	1-MG	2-MG	1-MG	2-MG	1-MG	2-MG	1-MG	2-MG	1-MG	2-MG
40	39·53	7·04	55·1	4·80	43·24	4·86	42·50	6·89	57·45	0·33	70·39	2·21
60	44·50	5·85	55·16	4·64	47·5	8·22	45·08	9·68	58·05	0·15	60·58	8·11
120	39·66	5·06	46·29	3·72	43·66	11·41	43·01	6·78	47·28	0·92	49·14	6·41
180	35·97	3·92	40·84	nil	38·88	5·94	36·46	9·84	39·18	1·97	44·00	1·01
225	32·51	5·08	33·37	nil	35·23	6·14	33·07	9·09	34·16	0·04	33·94	1·08

* MG represents monoglyceride.

work on the esterification of lauric, myristic, palmitic, stearic, oleic and linoleic acids with glycerol was undertaken to clarify the question of the formation of 2-monoglyceride in the esterification reaction mixture.

Fatty acid, purified by low-temperature fractionation, was made to react with an equimolar quantity of glycerol at a temperature of 180 ± 1° C., in an inert atmosphere of nitrogen, in the presence of potassium carbonate (0·1 per cent on the weight of fatty acid) as catalyst. Stirring was continued throughout the reaction.

Samples of 1–2 gm. were drawn at intervals of 40, 60, 120, 180 and 225 min. and these were at once treated with 2 drops of 85 per cent phosphoric acid which 'killed' the potash soap catalyst. The mass was dissolved in excess ether and the ethereal extract of the fatty matter washed with cold saturated sodium sulphate solution several times, 5 per cent potassium carbonate solution and finally with water. The washed ethereal solutions were dried overnight with anhydrous sodium sulphate, filtered and evaporated to yield the purified glycerolysed product.

1-Monoglyceride and total monoglyceride content of the samples were determined by Pohle, Mehlenbacher and Cook's method[7], as modified by Handschumaker and Linteris[8], and Martin's perchloric acid isomerization procedure[9] respectively; the difference between total- and 1-monoglyceride content represented the 2-monoglyceride content. In a separate set of samples drawn simultaneously from the esterification mixture, the percentage of free fatty acid was determined. The results are given in terms of the particular fatty acid with which the experiment was performed. Expressing the percentage of free fatty acid in the mixture at a time t as At ($A_0 = A_t$, where $t = 0$, A_0 corresponds to 0·5 mole-fraction) and denoting $\frac{A_t}{A_0} \times 100$ by B, the decrease in the values of A_t and B with time (as also a plot of log B versus log t) would indicate extent of esterification at any particular time. The observations are noted in Table 1 and Fig. 1.

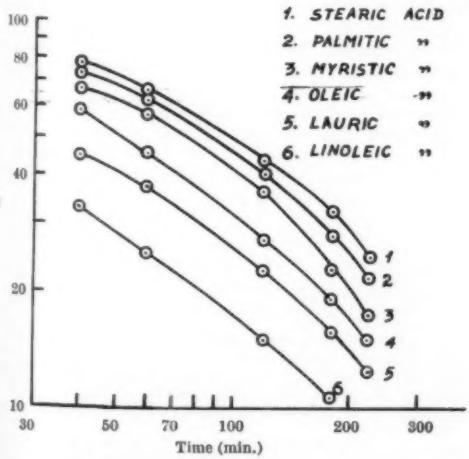

Fig. 1

The results clearly show that 2-monoglyceride constitutes a considerable proportion in the reaction products at various stages. Although it may be true, as suggested by previous workers[4,6], that 2-monoglyceride is prone to complete isomerization to 1-monoglyceride at higher temperature, this certainly does not hold good at or below 180° C. It would, therefore, be erroneous to report 1-monoglyceride as total monoglyceride, especially in esterification or glycerolysis reactions (as has been done in the current literature). Ignoring the presence of even a small amount of 2-monoglyceride would lead to wrong computation of the diglyceride content (the hydroxyl value being contributed not only by 1-monoglyceride and diglyceride but also by 2-monoglyceride); and hence also of the triglyceride content in the product obtained from esterification or glycerolysis reaction.

The rate of decrease of free fatty acid in the mixture indicates that the reactivity of fatty acids is greater with low molecular weight and unsaturated fatty acids. This reactivity is apparent, however, as the entire amount of fatty acid is not in a homogeneous phase with glycerol; low molecular weight and unsaturated fatty acids being relatively more miscible in glycerol[10], these react more quickly than the rest. The results obtained are not in full agreement with the observations of Toyama *et al.*[1], but they agree with those of Bhattacharyya and Hilditch[2].

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BIOCHEMISTRY

A Metabolite of 'Carbromal'

'Carbromal' (α-bromo diethylacetyl urea) is in widespread use as a sedative and hypnotic. None of its metabolites has, as yet, been fully characterized.

I recently isolated diethylacetyl urea from the intestine contents of a woman who was taking a proprietary drug containing 'Carbromal' 240 mgm. and 'Bromvaletone' 80 mgm. The method of isolation was that using acid ammonium sulphate, described by Nickolls[1]; 5 mgm. of the ureide crystallized readily from the neutral, ether-soluble fraction. Purification was by sublimation at 10^{-2} mm. at 100° C. It was at first thought that the product was α-ethyl crotonyl carbamate, which was noted as a product of the reaction of sodium hydroxide on 'Carbromal' by Newberry[2]. Both these compounds and their mixture melted within 5 deg. (200–205°); but because of their rapid sublimation these determinations were inadequate. Infra-red spectroscopy clearly differentiated the compound from α-ethyl crotonyl carbamate and from 'Sedormid' (allyl *iso*propyl, acetylurea). As a result of an examination of the spectra of several compounds in the series a specimen of diethylacetyl urea was prepared by hydrogenation of α-ethylcrotonylcarbamate (Dr. S. Trippett). After sublimation its spectrum in 'Nujol' was identical with that of the compound isolated from the intestines (Fig. 1). It seems probable that this is the compound observed by Turner[3].

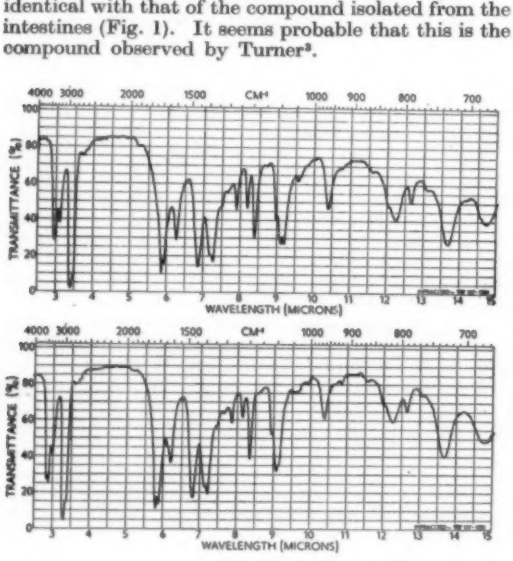

Fig. 1. The top curve shows the spectra of diethyl acetyl urea; the bottom curve is that of the crystals from the intestines

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Synthesis of Nicotinic Acid Nucleotides

Nicotinic acid nucleotide (5′-phospho-β-D-ribofuranosylpyridinium-3-carboxylate) and its adenylyl derivative, adenine-nicotinic acid dinucleotide, have recently been identified as intermediates in the biosynthesis of pyridine nucleotide coenzymes[1]. These compounds have been found in fungi[2,3], and the mononucleotide has been isolated in small amounts from yeast[4]. Both nucleotides have also been obtained in very low yields by enzymic modification of adenine-nicotinamide dinucleotide (coenzyme I)[2,3,5].

It has now been found that nicotinamide nucleotides, which are commercially available, may be conveniently converted into nicotinic acid nucleotides by treatment with nitrous anhydride. Preliminary work showed that nitrous acid or hydrogen chloride in amyl nitrite deaminates adenine nucleotides to hypoxanthine nucleotides, but does not liberate the amide nitrogen from nicotinamide nucleotides.

β-Nicotinamide nucleotide was suspended in dry acetic acid (10 ml. per millimole) and kept at −10° while an equal volume of nitrous anhydride[6] was condensed in the reaction vessel. The suspension was then kept at 10–15° for 1 hr. After removal of volatile material in a stream of dry air, the reaction products were applied to a column of 'Dowex-1-formate' and the nicotinic acid nucleotide was recovered by elution with formic acid[1]. The nucleotide was identified by electrophoresis, ion-exchange chromatography and paper chromatography, and by spectrophotometry in the presence and absence of cyanide[1,2,4].

When coenzyme I was treated with nitrous anhydride in acetic acid both amide and amine nitrogens were displaced, and hypoxanthine-nicotinic acid dinucleotide was obtained. This dinucleotide was quantitatively hydrolysed to nicotinic acid nucleotide and hypoxanthine nucleotide (inosine-5′-phosphate) by nucleotide pyrophosphatase from potato.

With adenosine triphosphate and magnesium ion at *p*H 7·6 in the presence of highly purified nicotinamide nucleotide-adenylyl transferase (coenzyme I pyrophosphorylase) from pig liver nuclei the nicotinic acid nucleotide was quantitatively converted into its adenylyl derivative. This derivative was likewise identified by the physical methods described above, and further by its conversion into coenzyme I in the presence of glutamine, adenosine triphosphate, magnesium ion and coenzyme I synthetase from pig liver cytoplasm. The coenzyme I was identified by reduction with ethanol and alcohol dehydrogenase from yeast.

Nicotinic acid nucleotide obtained in this way is a competitive inhibitor of enzymic adenylyl transfer from adenosine triphosphate to β-nicotinamide nucleotide, and is evidently identical with the natural coenzyme precursor.

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Proteolysis in Stored Serum and its Possible Significance in Cell Culture

ALMOST all the mammalian cell lines grown in continuous culture require the presence of a small amount of serum protein, usually supplied as whole or dialysed serum. The role of that protein, in an otherwise chemically defined medium[1], is not yet known. It is used to an insignificant degree for the synthesis of cell protein[2]; and although it is reported to be necessary for the adhesion and flattening of the cells on the glass surface[3,4], it is no less essential in suspension cultures. It is here shown that serum protein, presumably because of associated enzymes[5], undergoes proteclysis on storage at 5° C. or during incubation to yield free amino-acids at a rate which may be significant in certain types of experiments. The amino-acids released are themselves not growth-stimulating since they are already supplied in the medium or are formed from glucose and glutamine. However, other breakdown products of the serum protein, or compounds bound to it and released by its degradation, may be the essential growth factors supplied by serum.

Pooled human serum protein was freed of small molecules by two different procedures. Dialysed serum was prepared by dialysis of serum on a rocking dialyser against running water at 5° C. for 24 hr.; sodium chloride was then added to give a concentration of 0·85 per cent. Gel-filtered serum was prepared by the methods developed by Porath and Flodin[6]. A 3·5 cm. × 40 cm. column (400 ml.) of dextran gel ('Sephadex *G*–50') was poured and washed with 500 ml. of 0·85 per cent sodium chloride. 100 ml. of serum was allowed to pass into the column and elution was continued with 0·85 per cent sodium chloride. The first 100 ml. of effluent was discarded. The following 120 ml., which contained all the protein, was collected. Small molecules from the serum did not appear in the effluent until another 80 ml. of effluent (300 ml. total) had passed. The column was washed with an additional 500 ml. of salt solution before being re-used.

The dialysed and gel-filtered serum preparations were sterilized by filtration through sintered-glass filters and stored at −20°, 5°, or 37° C. for various times. The free amino-acids were determined on samples which were deproteinized with picric acid[7] and lyophilized. Portions of the dissolved residue representing 2–4 ml. of serum were analysed on an automatic amino-acid analyser[8].

The results are presented in Table 1. An analysis of the free amino-acids in a pooled sample of fresh whole serum is included for comparison (column 1). It is apparent from these results that dialysed serum undergoes proteolysis at an appreciable rate even when stored at 5° C. (column 2). After 11 days the amino-acid levels in some cases were as high as those found in fresh whole serum, averaging about 30 per cent. Gel-filtered serum was considerably more stable. After 30 days at 5° C. (column 5), the amino-acid concentrations averaged about a third those observed in dialysed serum after 11 days at the same temperature. The higher stability of the gel-filtered serum was even more evident at 37° C. In an experiment employing one sample of pooled serum (columns 3, 4, 6, and 7), the free amino-acid level in dialysed serum after 5 days was more than half that in whole serum, while in gel-filtered serum it averaged only a tenth. After 28 days the amino-acid levels in dialysed serum had increased on average by nearly 60-fold, but less than 4-fold in gel-filtered serum. At −20° C. only negligible increases were observed in serum prepared by either procedure.

Repeat experiments gave essentially the same results as those reported in Table 1 with some variation between individual lots of serum. In some instances the occurrence of other reactions in addition to proteolysis was indicated by the appearance of amino-acids not present in proteins. The concentrations of citrulline, α-aminobutyric acid, and ornithine which were found in stored or incubated serum are listed in Table 1. The presence of other unidentified ninhydrin-reacting compounds was also indicated by the chromatograms.

It is abundantly clear that dialysed serum stored in the refrigerator cannot be considered to be free of amino-acids. The problem is less serious with gel-filtered serum. The reason for the more rapid breakdown of dialysed serum protein is not clear. It may be related to more efficient removal by the gel-filtration procedure of small molecules necessary as co-factors.

In order to examine the rate of proteolysis under cell culture conditions, dialysed and gel-filtered serums were incorporated at the 10 per cent level

Table 1. CONCENTRATION OF FREE AMINO-ACIDS IN SERUM (mM)

Amino-acid	Whole serum	Dialysed serum*			Gel-filtered serum†			10 per cent serum‡ 37°, 3 d.	
		5° 11 d.	37° 5 d.	37° 28 d.	5° 30 d.	37° 5 d.	37° 28 d.	Dialysed	Gel-filtered
Aspartic acid	0·017	0·019	0·045	2·11	0·013	0·004	0·048	0·055	0·005
Threonine	0·156	0·064	0·083	0·24	0·017	0·016	0·059	0·031	0·005
Serine	0·149	0·051	0·132	0·01	0·057	0·031	0·092	0·062	0·010
Glutamic acid	0·076	0·071	0·220	9·43	0·023	0·028	0·109	0·083	0·010
Citrulline	0·034	0·019	0	0·87	0	0	0	0	0
Proline	0·273	0·029	0·080	7·42	0	0·014	0 040	0·029	0
Glycine	0·246	0·063	0·129	4·97	0·027	0·019	0·054	0·064	0·010
Alanine	0·413	0·078	0·256	11·96	0·030	0·038	0·102	0·068	0·019
Cystine	0·053	0	0	2·52	0	0	0	0·010	0·005
α-Aminobutyric acid	0·011	0	0	0·29	0	0	0	0	0
Valine	0·254	0·029	0·088	9·50	0	0 014	0·056	0·023	0·005
Methionine	0·021	0·002	0·029	2·92	0	0·006	0·017	0·010	0
*iso*Leucine	0·071	0·005	0·022	2·25	0·031	0·007	0 017	0·015	0·005
Leucine	0·132	0·062	0·180	11·66	0·039	0·032	0·108	0·057	0·015
Tyrosine	0·046	0·010	0·036	4·07	0	0·007	0·029	0·015	0
Phenylalanine	0·057	0·029	0·059	7·92	0 018	0·007	0·040	0·038	0
Ornithine	0·111	0·015	0·014	4·97	§	0·014	0·053	0	0
Lysine	0·174	0·048	0·112	15·75	§	0·020	0·078	0·044	0·010
Histidine	0·096	0·024	0·043	0·52	§	0·007	0·023	0·024	0·005
Arginine	0·075	0·088	0·106	0·17	§	0·011	0·023	0·053	0·015

* Dialysed 24 hr. against running water. †, Small molecules removed by filtration through 'Sephadex *G*-50' (ref. 6). ‡ Serum added to a medium (ref. 1), which was complete except for free amino-acids; concentrations calculated for undiluted serum. § Not analysed.

into a medium[1] which was complete except for the essential amino-acids and which was then incubated at 37° C. for 3 days. Free amino-acids appeared at about the same rate as in whole serum. With gel-filtered serum the final amino-acid concentrations were all less than 0·02 m*M* and averaged about 0·005 m*M*, calculated for undiluted serum. Dialysed serum released amino-acids at about six times this level.

In experiments in which the essential amino-acids are added to the medium in concentrations greater than about 0·05 m*M*, the additional amounts released by proteolysis would probably not be significant. At lower levels, however, the free amino-acids contributed by serum protein could have an appreciable effect, particularly in the case of dialysed serum. In one experiment, when cells were grown in a presumably valine-free medium supplemented with 5 per cent dialysed serum and 0·002 m*M* valine labelled with carbon-14, the specific activity of the valine residues in the cell protein was only 23 per cent that of the precursor amino-acid, the rest deriving from the protein (Eagle, H., and Piez, K. A., unpublished work). Similarly, in the case of the nutritionally non-essential amino-acids, which are synthesized by the cell from glucose and glutamine, the contaminating amino-acids derived from serum proteolysis may introduce a large error in isotope labelling experiments.

Although we have analysed only the free amino-acids formed by proteolysis, it is evident that large numbers of various-sized polypeptides must also be formed, and that some of these may be utilized by cells in culture[9]. Work is in progress in an attempt to evaluate this aspect of serum proteolysis. It is possible that such peptides may be an important and perhaps necessary contribution by serum protein, particularly in the light of the demonstration[10] that dialysable substances formed by the action of a dialysed pancreatic extract or crystalline proteolytic enzymes on serum protein can be substituted for the native serum protein in cell growth.

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Distribution of Guanylic Acid Residues in Tobacco Mosaic Virus Nucleic Acid

The mode of arrangement of purine and pyrimidine nucleotides in tobacco mosaic virus nucleic acid was studied using the actions of pancreatic ribonuclease and micrococcal phosphodiesterase. This work showed that in tobacco mosaic virus nucleic acid the purine nucleotides and pyrimidine nucleotides occur in 'clusters' of two or more and also 'singly' alternating with each other. The extension of these studies to nucleic acids of three viral strains (*TMV*, *HR* and *M*) revealed for the first time significant differences in the arrangement of nucleotides in each of their molecules[1–5]. Additional information on the distribution of guanylic acid residues in tobacco mosaic virus nucleic acid, obtained by the action of an enzyme specific to secondary phosphate esters of guanosine 3′-phosphates, is given in the present communication.

Sato and Egami[6] recently reported the isolation and properties of a ribonuclease, present in taka diastase, which specifically cleaves the secondary phosphate esters of guanosine 3′-phosphates. This important discovery provided a much-desired additional tool for structural studies in ribonucleic acids. This enzyme has been purified in this laboratory using a modified procedure, the details of which will be reported at a later date. My preparations have properties similar to those described by the above investigators. The *p*H optimum in 0·1 *M tris* buffer was found to be at 7·4. When assayed according to the procedure described earlier[7] my preparations were found to contain 6×10^6 units of enzyme per mgm. of protein. This enzyme is specific in that it slowly converts guanosine 2′ : 3′-cyclic phosphate to guanosine 3′-phosphate exclusively. It degrades ribonucleic acid giving rise to monoguanylic acid (guanosine 2′ : 3′-cyclic phosphate, Gp! and guanosine 3′-phosphate, Gp) and oligonucleotides terminated by guanylic acid (Gp! and Gp). From these results it can be concluded that this enzyme cleaves the ribonucleic acid via transphosphorylation giving rise to guanosine 2′ : 3′-cyclic phosphates and oligonucleotides terminated by them and these are slowly converted to guanosine 3′-phosphates. Its mechanism of cleavage via transphosphorylation is reminiscent of that of leaf ribonuclease[8] even though the specificities of the two enzymes are different. This enzyme will be referred to here as taka ribonuclease-I.

The occurrence of polyguanylic acid segments in tobacco mosaic virus nucleic acid was established using the action of taka ribonuclease-I. 2 mgm. of tobacco mosaic virus nucleic acid, prepared according to the heat denaturation procedure[9], was dissolved in 0·4 ml. of 0·2 *M tris* buffer at *p*H 7·4 containing 4×10^{-3} *M* ethylenediamine tetraacetic acid. To this was added 0·1 ml. of taka ribonuclease-I (about 1,000 units, a large excess to ensure complete digestion) and a drop of chloroform to prevent microbial growth. The mixture was incubated at 37° for 18 hr. In the complete digest monoguanylic acid (Gp! and Gp) was found to an extent of 26·9 per cent of total guanylic acid residues in tobacco mosaic virus nucleic acid. This was calculated using the base composition data reported earlier[10]. Since the enzyme is specific to guanosine 3′-phosphate linkages, the minimum requirement for a monoguanylic acid to appear in the digest is the occurrence of at least two guanylic acid residues linked together. From this it can be concluded that about 26·9 per cent of total guanylic acid residues in tobacco mosaic virus nucleic acid occur in 'clusters' of two or more, $Gp(Gp)_n$, where $n = 1$ or more.

The average length of the larger fragments produced by the digestion of tobacco mosaic virus nucleic acid with taka ribonuclease-I was determined as follows: 9·2 mgm. tobacco mosaic virus nucleic acid, prepared according to heat denaturation procedure[9], were dissolved in 1 ml. of 0·04 *M tris* buffer at *p*H 7·4 containing 10^{-3} *M* ethylenediamine tetraacetic acid and to this was added 0·1 ml. of taka ribonuclease-I (10,000 units) and a drop of chloroform. The mixture

was incubated at 37° for 18 hr. At the end of the reaction time, the digest was transferred to an ice-bath and to this was added with mixing cold 50 per cent trichloroacetic acid. The final concentration of this acid in the mixture was 6 per cent. This was held at 0° for 30 min. and centrifuged at 4° for 15 min. at 2,500 r.p.m. The precipitate was washed with 0·1 ml. cold 6 per cent trichloroacetic acid and this was followed by one washing with 0·5 ml. of cold absolute alcohol. The washed precipitate was dissolved in 2 ml. of 0·02 *M* borate buffer at *p*H 8·6. This was then transferred to a dialysis bag and dialysed at 4° C. against 0·02 *M* borate buffer at *p*H 8·6 for 8 hr. with continuous stirring. The purine and pyrimidine composition[11] of the dialysed preparation was found to be as follows: guanine, 1·00; adenine, 2·17; cytosine, 1·34 and uracil, 1·53. The amount of guanylic acid present in the entire dialysed material was about 29·3 per cent of the total guanylic acid residues in tobacco mosaic virus nucleic acid. This preparation is a heterogeneous mixture of several polynucleotides terminated by guanylic acid. From the purine and pyrimidine composition and the specificity of the enzyme used, it can be suggested that they average about six nucleotides in length, $(Xp)_5Gp$ (where *X* is adenosine, cytidine and uridine), and these are all preceded by a guanylic acid residue in the intact tobacco mosaic virus nucleic acid.

In addition to monoguanylic acid in the complete digest of tobacco mosaic virus nucleic acid, three dinucleotides, cytidylyl-guanylic acid, uridylyl-guanylic acid and adenylyl-guanylic acid and several tri-, tetra-, etc., nucleotides terminated by guanylic acid were isolated. Full details of this investigation will be published elsewhere.

I am indebted to Dr. W. M. Stanley for his encouragement and support of this research.

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Note added in proof. Guanylic acid residues in a nucleic acid, in addition to occurring in 'clusters' of two or more, $(Gp)_nGp$, can also occur distributed in two other ways: preceded by pyrimidine nucleotides, cytidylic and uridylic acids, $(Pyp)_nGp$; and preceded by adenylic acid, $(Ap)_nGp$. Evidence for this type of distribution in tobacco mosaic virus nucleic acid was obtained by exhaustively digesting the nucleic acid with both pancreatic ribonuclease and taka ribonuclease-I[12]. About 46·4 per cent and 28·4 per cent of total guanylic acid residues in tobacco mosaic virus nucleic acid were found to occur as $(Pyp)_nGp$ and $(Ap)_nGp$ respectively.

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Biosynthesis of Damascenine in *Nigella damascena* L.

Damascenine is present in the seeds of *Nigella damascena* L. to the extent of about 0·1–0·3 per cent (dry weight). The majority of the alkaloid is present in the testas. The seeds of other *Nigella* species hitherto investigated possess only traces of the base.

In the past few years Tyler[1] as well as Leete, Marion and Spenser[2] have dealt with the physiology and biosynthesis of damascenine. In order to clarify further the problem of the origin of damascenine in the plant we have studied its ontogenesis and its site of formation; experiments involving the feeding of labelled precursors have also been undertaken.

Damascenine was isolated from the plant material in the usual way and characterized by paper chromatography: circular paper-chromatographic technique was employed using Schleicher and Schüll chromatographic paper 2043 *b*, which was impregnated with phosphate buffer (*p*H 8); methanol served as the mobile phase; detection of damascenine (R_F 0·79) was achieved by its characteristic blue fluorescent property under filtered ultra-violet light. While with the help of Dragendorff's reagent (modified after Munier) quantities of more than 30 μgm. of damascenine could be identified on the filter, it required only as low as 1–2 μgm. of the alkaloid to be readily detected under ultra-violet light. Damascenine loses its fluorescing property in acid medium. In order to determine it quantitatively, the base was eluted from the filter with 96 per cent alcohol and with the aid of a reference curve the alcoholic solution was fluorometrically assayed for its damascenine content.

During the course of natural development of the cultivated plants, the damascenine content of different organs of a large number of *Nigella damascena* plants was determined at definite intervals. The seedlings, which were grown in sand, were found to be free from damascenine. This confirms the observations already made by Tyler[1]. In the earlier stages of development a very low damascenine content was found in the roots, stems, leaves and flowers; but this gradually increased with the progressive growth of the plants. Beginning with the ripening of the seeds, an enormous increase of damascenine content was noted, and this attained its maximum when the seeds reached maturity. At this stage of development the following values for damascenine content (dry weight) were determined: seeds 0·15 per cent, leaves 0·013 per cent, stems 0·0001 per cent and roots 0·0002 per cent.

In order to locate the site of damascenine synthesis in the plant, excised roots were grown under sterile conditions. Robbins's culture medium was employed, which consisted of 1·5 per cent sucrose, inorganic salts and vitamins, supplemented by the addition of 7 mgm. of arginine per litre (all dissolved in three-times distilled water). The roots were grown in Erlenmeyer flasks, each of which contained 20 ml. of the culture medium. The roots exhibited good growth when they were incubated in dark at 25° C. After an incubation period of 40 days there was on the average 12 mgm. of dried root mass found in each flask. Both the roots and the culture medium were at the end of the incubation period deep yellow in colour. The yellow colouring matter could be readily extracted from the culture medium by shaking it with chloroform (yield: 16 μgm. per

ml. culture solution). Paper-chromatographic investigations of the colouring matter in different solvent systems showed that it consisted of at least two different components, one of which exhibited a yellow, and the other a greenish-yellow, fluorescence in ultra-violet light. Further characterization of these compounds is in progress. Damascenine was present neither in the excised roots nor in the culture medium. Hence it is inferred that the isolated roots are not capable of biosynthesizing damascenine.

Grafting experiments were undertaken to identify the site of the biosynthesis of damascenine in the plant; and to this effect *Nigella damascena* L. was reciprocally grafted on to *Nigella sativa* L. and *Nigella arvensis* L., which are practically free of damascenine. The heteroplastically grafted specimens showed a comparatively smaller longitudinal growth than the ungrafted plants. However, they flowered and developed fruits and ripe seeds in the normal manner. By considering the results derived from a number of analyses carried out on these grafts, it can be established that the roots of *Nigella damascena* do not represent the site of biosynthesis of damascenine. This is in close agreement with the results of the investigations on sterile root cultures described here. It has been found instead that the shoot of *Nigella damascena* is capable of independently synthesizing the alkaloid. It is, however, not known whether the occurrence of damascenine in traces in *Nigella arvensis* as well as in *Nigella sativa* is somehow connected with degradation of damascenine in the plant. Investigations on this problem employing the use of suitably labelled damascenine are under way.

Young leaves of *Nigella damascena*, the stalks of which were dipped in water, were allowed to assimilate in light in an atmosphere of carbon-14 labelled carbon dioxide for a week. 31·2 per cent of the total activity used in the experiment was taken up by the leaves. Damascenine isolated from the dried leaves was purified by paper-chromatographic technique and with the help of radioautography it was found to be radioactive. The isolated damascenine contained 0·256 per cent of the activity taken up by the leaves. In a similar experiment using excised unripe fruits there was no trace of radioactive damascenine to be detected in the fruits chromatographically after having allowed them to stay for two weeks in an atmosphere of labelled carbon dioxide. It may be mentioned here that this is the stage at which the intact plants begin to biosynthesize damascenine at a rapid rate. Hence it can be concluded from these surprising results that the leaves are capable of participating in the biogenesis of damascenine.

As damascenine is a derivative of anthranilic acid, we therefore undertook feeding experiments with labelled anthranilic acid ($-{}^{14}COOH$). For this purpose the plants of *Nigella damascena* were aseptically grown in a culture solution containing inorganic salts. With the appearance of fruits on the plants the labelled anthranilic acid was fed (*a*) by injection into the stem, and (*b*) to the roots by adding it to the culture solution. After having fed the labelled compound, the test plants were further allowed to grow aseptically for about four weeks, at the termination of which they possessed ripe fruits and seeds; the plants were harvested at this stage and damascenine extracted from different organs was found to be inactive. This means that ^{14}C-carboxyl-labelled anthranilic acid, under these experimental conditions, was not utilized by *Nigella damascena* plants as a precursor for the biosynthesis of damascenine, or it might have been used up only after the loss of the activity by decarboxylation. Further work in this direction is being continued. The full details of the results obtained will be published elsewhere.

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Severe Distortion by 5-Bromouracil of the Sequence Characteristics of a Bacterial Deoxyribonucleic Acid

The statement is often encountered in the literature that one or another purine or pyrimidine analogue, not found in the cell normally, can, when it is incorporated into a deoxy- or ribo-nucleic acid, partly replace one of the natural nucleic acid constituents. For example, 5-bromouracil is said to replace some of the thymine in deoxyribonucleic acid, or 5-fluorouracil some of the uracil of ribonucleic acid; and this view is even applied to minor constituents occurring in Nature, 5-methylcytosine replacing some of the cytosine, pseudouridine some of the uridine. Such statements are derived, implicitly, from the complementariness principles formulated in this laboratory for deoxyribonucleic acid[1] and ribonucleic acid[2]. But what actually is meant by replacement? If, as is the case, the molar sum of the 6-aminopyrimidines cytosine and 5-methylcytosine equals the molar concentration of the 6-ketopurine guanine in the deoxyribonucleic acid of rye germ[3], does this signify that every cytosine molecule along the polymer chain has an equal chance of being 'replaced' by a methylcytosine molecule? We have recently discussed the evidence for this not being the case[3].

The procedure for the study of the pyrimidine distribution in deoxyribonucleic acids, which in previous instances permitted differentiation between otherwise indistinguishable nucleic acid preparations from different cellular sources[4,5], has again been applied in the present investigation. In a previously studied case, the deoxyribonucleic acid of rye germ, it could be shown that a normal minor constituent, 5-methylcytidylic acid, does not replace the related 6-amino nucleotide cytidylic acid in a random fashion[3]. One of the notable features was, in fact, the much greater tendency of methylcytidylic than of cytidylic acid to be interjacent between purine nucleotides, that is, to occur as what we have termed a solitary unit[5].

It was, consequently, of interest to study the nucleotide pattern of a deoxyribonucleic acid that

had been forced to admit a minor, abnormal component, 5-bromouracil[6], ostensibly in the place of thymine[7,8]. In such systems, no obvious cellular mechanism of regulation, except death itself, can be discerned that would limit the extent of incorporation of the halogenated pyrimidine, whereas the complement of methylcytosine, and presumably also of other natural satellites, appears to be fixed rigidly by the cell[3,9].

The purpose of the present work was to ascertain whether, in the deoxyribonucleic acid of an *E. coli* strain requiring thymine, bromouracil really replaces thymine or whether the cell possesses a selection mechanism capable of distinguishing, to some extent, between two 6-ketopyrimidines, the normal thymine and the fraudulent bromouracil; a distinction demonstrated before in the case of the two normal 6-aminopyrimidine constituents of rye germ nucleic acid.

The thymine auxotroph of *E. coli* strain I employed in previous studies[8] was grown for 18 hr. at 37° on a slightly modified synthetic medium[10] to which 2 μgm./ml. of thymine had been added either alone (prep. 1) or together with 10 μgm./ml. of 5-bromouracil (prep. 2). The deoxyribonucleic acid specimens were isolated by a modification of a procedure employing the treatment of the cells with a buffered phenol–water system[11].

Table 1. Composition of Deoxyribonucleic Acid of *E. coli* grown in the Absence and Presence of 5-Bromouracil

Preparation No.	Growth supplements	Moles constituent per 100 moles total					Molar ratios	
		A	*G*	*C*	*T*	*B*	$\frac{6\text{-}Am}{6\text{-}K}$	$\frac{A}{T}$ or $\frac{A}{T+B}$
1	*T*	24·8	25·0	25·5	24·8	—	1·01	1·00
2	*T* + *B*	25·3	24·3	25·1	16·4	9·0	1·01	1·00

A, adenine; *G*, guanine; *C*, cytosine; *T*, thymine; *B*, 5-bromouracil.

The composition of the nucleic acid preparations is shown in Table 1. It will be seen that, in a formal sense and as regards its total abundance, bromouracil can indeed be said to have occupied more than one-third of the places originally held by thymine.

Table 2. Frequency of Solitary and Coupled Pyrimidine Nucleotide Units

Preparation No.	Moles per 100 gm. atoms of total nucleic acid phosphorus				
	pTp	*pBp*	*pCp*	*pCpCp*	*pCpTp*
1	3·82	—	1·78	0·37	1·13
2	3·01	2·96	3·60	0·58	1·38

pTp, *pBp* and *pCp* designate the nucleoside 3′,5′-diphosphates indicative of solitary units, *pCpCp* and *pCpTp* the dinucleoside triphosphates. Compare Table 1 for other explanations and a previous paper (ref. 3) for the description of designations and techniques.

An entirely different aspect emerges, however, when individual positions of the pyrimidine nucleotides within the polymer chains are examined. The findings summarized in Table 2 were obtained by employing stage I of a previously described hydrolysis procedure (30 mgm. deoxyribonucleic acid treated with 1·5 ml. 0·1 *M* sulphuric acid at 100° for 30 min.)[4]. The separation and estimation of the fragments followed, essentially, the methods using chromatography on exchange resins and filter paper that have been described before[3]. In this manner, a fairly complete account can so far be given of the solitary pyrimidine nucleotides, isolated in the form of the corresponding nucleoside 3′,5′-diphosphates, and of some of the 'coupled' units, that is, two adjacent pyrimidine nucleotides flanked on both sides by purine nucleotides.

Had an unspecific replacement or displacement of thymine by bromouracil occurred, this would have been reflected in an equal relative utilization of both pyrimidines for common sequences, such as purine—thymine—purine and purine—bromouracil—purine. A glance at Table 2 will show that this is not at all the case. The reorganization of the nucleotide pattern of the deoxyribonucleic acid, brought about by the intrusion of the bromouracil, is so drastic that one receives the impression of dealing with two largely different populations of polymers[12]. In the cells grown in the presence of the halopyrimidine, the nucleic acid contains almost twice as many solitary pyrimidine units as does the normal preparation; the frequency of purine—cytosine—purine sequences has doubled and even the relative contribution of thymine to the corresponding units has increased; moreover, nearly one-third of the incorporated bromouracil has been segregated between purines in the form of solitary units. On the other hand, the concentration of the coupled unit purine—cytosine—thymine—purine has been comparatively little disturbed.

A fuller account of this and related work will be submitted later. For the time being, one must conclude that it cannot be stated that thymine has, in a specific sense, been 'replaced' by 5-bromouracil. One gains the impression that, after the selection of equal numbers of 6-aminopurines and 6-ketopyrimidines, on one hand, and of 6-ketopurines and 6-aminopyrimidines on the other, a second mechanism—an exclusion principle, as it were—comes into play, so that certain neighbours are tolerated and others not, or rarely.

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A Method for studying Amino-Acid Activation in Crude Tissue Extracts

There is now considerable evidence that the first step in protein synthesis involves amino-acid activation[1]. Using the methods of pyrophosphate exchange into adenosine triphosphate, and of hydroxamate formation, activation of all the common amino-acids, added singly, has been demonstrated in plant[2], animal[3] and bacterial[4] extracts, although the entire complement of activities is not usually detectable in most preparations[3]. Both these assay methods depend on differences observed in the presence and absence of added amino-acid, and difficulties in interpretation of results arise when applied to crude preparations in which the endogenous level of amino-acids is high. On the other hand, because of the lability of amino-acid-activating enzymes to procedures such as dialysis[5], any purification procedure to overcome this difficulty carries with it the danger of inactivating part of the enzyme systems being investigated.

In connexion with work on protein synthesis being carried out here, the need was felt for a method of analysing the amino-acid activation pattern in crude cell extracts, and, furthermore, it seemed advantageous that this should be done in the presence of the complex mixture of amino-acids necessary for protein synthesis. The latter consideration seemed especially important in view of the reported competition effects between structurally related amino-acids for the amino-acid-activating enzymes[4]. This communication describes a method for the quantitative measurement of the total amino acyl hydroxamates formed in the presence of a complex mixture of amino-acids and a further chromatographic procedure for the identification of at least 15 of the individual amino acyl hydroxamates. The procedure is unaffected by the endogenous level of amino-acids in the extracts. The application of the method to crude rat liver preparations as a test system is described here.

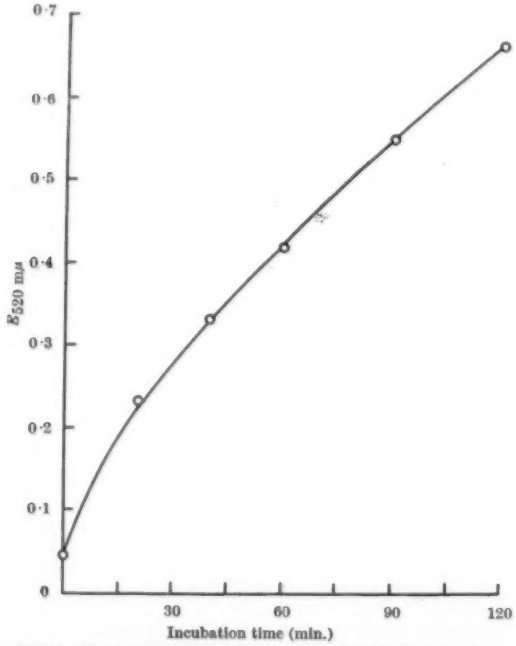

Fig. 1. Progress curve for amino acyl hydroxamate formation by crude 105,000*g* supernatant fraction of a rat liver homogenate. The complete system contained 25 μmoles K_2adenosine triphosphate, 50 μmoles magnesium chloride, 6,000 μmoles 'low salt' hydroxylamine, 75 μmoles L-amino-acid (mixture containing equimolar concentrations of the 15 amino-acids, alanine, arginine, glycine, histidine, *iso*-leucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine), 250 μmoles potassium phosphorylcreatine, 100 μgm. crystalline creatine phosphoryltransferase, and 1·5 ml. 105,000*g* supernatant. The volume of the reaction mixture was 5·0 ml. and the *p*H 7·5. Incubations were carried out at 37°

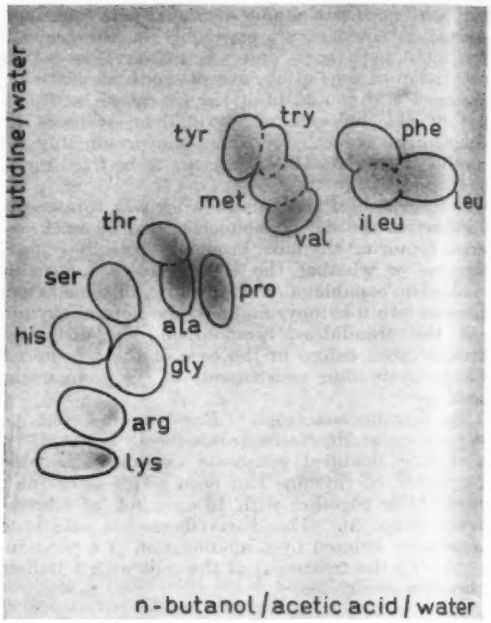

Fig. 2. Two-dimensional chromatogram of a mixture of 15 synthetic amino acyl hydroxamates

Rat liver was homogenized in a Potter–Elvehjem apparatus with 1·5 vol. of ice-cold 0·05 *M* potassium chloride and centrifuged at 105,000*g* for 60 min. Samples of the supernatant fraction were then added to reaction mixtures containing 15 amino-acids, 'low-salt' hydroxylamine, magnesium chloride, adenosine triphosphate and an adenosine triphosphate-generating system in a volume of 5·0 ml. After incubation, the reaction was stopped by the addition of 1·0 ml. of 60 per cent perchloric acid, and diluted with 6·0 ml. of water. The supernatant was collected by centrifugation and the residue washed with water. The combined supernatant and washings were adjusted to *p*H 7·5 with 3 *N* potassium hydroxide and the precipitate of potassium perchlorate filtered off. The filtrate was evaporated to dryness under reduced pressure at 37° and the residue stored overnight in a desiccator over concentrated sulphuric acid to remove residual hydroxylamine. The residue was extracted with 10 ml. of water and the extract adjusted to *p*H 5·0 with acetic acid. This solution was then passed through a column of 'Amberlite *IRC*-50', equilibrated at *p*H 5·0 with ammonium acetate buffer. After exhaustive washing with water, the column was eluted with 30 ml. of 2 *N* ammonium hydroxide and the eluate evaporated to dryness under reduced pressure at 37°. The residue was dissolved in 5·0 ml. of water and amino acyl hydroxamates determined by the method of

Schweet[6]. Control experiments showed that the column gave consistent 80 per cent recovery values with a mixture of 15 chemically prepared amino acyl hydroxamates. The incomplete recovery is not due to a loss of specific components, since material eluted from the column gave the same loss on recycling. The procedure does not permit the estimation of the hydroxamic acid derivatives of the dicarboxylic amino-acids but does have the advantage of eliminating aspartic and glutamic-ω-hydroxamates, both of which may be formed enzymically[7,8].

Fig. 1 shows a progress curve of amino acyl hydroxamate formation by a crude rat liver soluble fraction.

For the identification of the amino acyl hydroxamates present, the dried eluate from '*IRC*-50' columns, obtained as above, was extracted with a minimum amount of 90 per cent ethanol. The hydroxamate solution obtained was chromatographed in two dimensions on acid-washed Whatman No. 3 papers, run first in water-saturated 2 : 4-lutidine, followed by *n*-butanol–acetic acid–water (4 : 1 : 1). The spots were detected by spraying with 6 per cent ferric chloride in 0·2 *N* hydrochloric acid. For satisfactory results care must be taken that both ion exchange columns and chromatographic papers are metal-free. The resin was given a preliminary wash with ammonium versenate before use, and the papers were washed in 50 per cent acetic acid and then with water until acid-free[9]. In Fig. 2 the separation of a mixture of 15 synthetic amino acyl hydroxamates is shown. Fig. 3 shows the separation of the mixture of hydroxamates formed by rat liver. The identity of the hydroxamates of glycine, lysine, proline, serine and tyrosine was confirmed by the addition of reinforcing markers. The spots designated methionine and tryptophan were very faint and could not be identified with certainty. The separation is susceptible to overloading and this has been shown to be the reason for the distortion of the proline hydroxamate spot. In heavily loaded chromatograms a faint spot corresponding in position to the hydroxamate of histidine has also been observed.

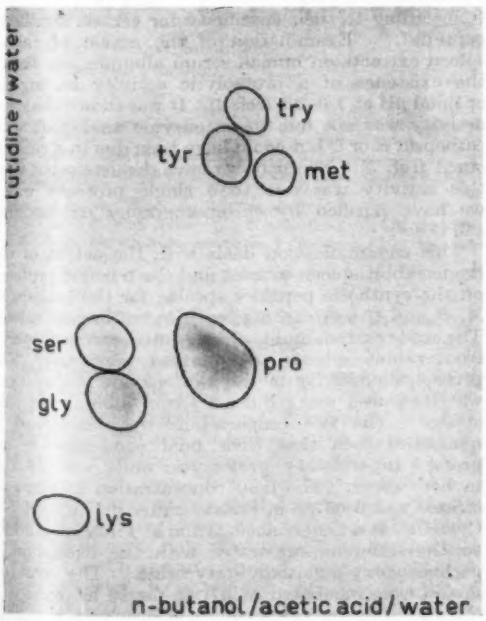

Fig. 3. Two-dimensional chromatogram of enzymically formed amino acyl hydroxamates. The hydroxamates were formed and isolated as described in the text

The procedure described here enables identification of at least 15 amino acyl hydroxamates formed simultaneously as a result of amino-acid activation in crude enzyme preparations. With suitable use of labelled amino-acids and carrier marker spots, the sensitivity could doubtlessly be considerably refined. It is also hoped that it may prove possible to apply quantitative methods of estimation to the individual components of the chromatograms.

Full details of this work will be reported later.

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Quantitative Isolation of Alkaloids from Plant Materials

The common methods of isolation of alkaloids in relatively pure forms from plant materials now available involve extraction by solvent partition and/or column chromatography. These methods are very cumbersome, emulsions usually occur, large volumes of solvents are required and the final products are very often impure.

The fact that ammonium reineckate forms insoluble derivatives with alkaloids is well known. The mechanism of the reaction between organic bases and ammonium reineckate has been studied, and the regeneration of the conjugate bases from their reineckates using ion-exchange resins has also been reported[1]. This suggests a method for the isolation of plant alkaloids as their insoluble reineckates from which the free alkaloids can later be regenerated by passing an acetone solution of these reineckates through an ion-exchange column. A detailed study of this method was undertaken in order to evaluate it as a means of recovering and determining alkaloids in both opium and nux vomica. The procedure yielded crystalline alkaloids even from opium, and the quantitative results obtained indicated a high degree of accuracy and precision. The basic technique can be applied to the isolation of alkaloids from other plant materials provided these alkaloids do not decompose on the ion-exchange column. This simple procedure promises to offer the first method known for the quantitative determination of the absolute amounts of the various alkaloids in opium. The quantitative results obtained for strychnine in nux vomica are very satisfactory, and a detailed report of this investigation will be published elsewhere.

Isolation of Opium Alkaloids. About 1 gm. of opium is treated with 50 ml. of 10 per cent hydro-

chloric acid for ½ hr. over a water-bath. The solution is filtered through a Whatman No. 1 filter paper and the residue washed with 3 ml. portions of warm dilute hydrochloric acid until the filtrate gave no precipitate with Mayer's reagent. 50 ml. of 2 per cent ammonium reineckate solution is added to the acid solution, cooled to 0° C. and filtered through a sintered-glass crucible with the aid of gentle suction. The precipitate is dissolved in acetone and calcium oxide is added to the acetone solution. A considerable amount of impurities is removed by the calcium oxide in this manner. This solution is filtered through a sintered-glass crucible, and the calcium oxide washed with acetone until the filtrate is colourless. The bright red-coloured acetone solution is then passed through an ion-exchange column of 'De-Acidite *FF*' which has previously been activated by passing a solution of 5 per cent sodium hydroxide and then washed with water until the eluate has a *p*H of 7–8. The colourless acetone eluate contains codeine, thebaine, papaverine and narcotine and the morphine is held back in the column. About 20 ml. of water is added to the acetone solution and concentrated to about 10 ml. The solution is made alkaline by the addition of sodium hydroxide and the alkaloids extracted from this solution with chloroform. Crystalline products are obtained on evaporation of the chloroform solution and tituration of the residue with 50 per cent ethanol.

The morphine is recovered from the column by first washing the column with water to remove the acetone and then with 10 per cent acetic acid solution. The acetic acid solution is concentrated to about 10 ml. and ammonium hydroxide is added until the solution is alkaline. The ammoniacal solution is extracted with 3 : 1 chloroform-*iso*butanol mixture and morphine is obtained upon evaporation of this solvent mixture.

Quantitative Determination of Papaverine in Opium. The chloroform extract containing codeine, thebaine, narcotine and papaverine is not evaporated but is extracted with 3 portions of 20 ml. of *N*/5 hydrochloric acid which removes the codeine and thebaine from the organic layer. The chloroform layer is transferred to a separating funnel and 50 ml. of *N*/5 hydrochloric acid is added to it. This mixture is shaken thoroughly and 30 ml. of 2 per cent ammonium reineckate solution is then added. The papaverine is determined by the method according to Lee and Farmilo[2,3]. The results are shown in Table 1. Satisfactory results were also obtained for narcotine. The procedures for the determination of codeine, thebaine and morphine are still under study.

Table 1. Determination of Papaverine in Opium

Amount of opium used (gm.)	Amount of papaverine obtained (mgm.)	Percentage present
1·082	15·1	1·40
1·084	15·4	1·42
1·160	16·0	1·38
1·070 and 10 mgm. papaverine	24·9	1·39

Isolation of Strychnine and Brucine from Nux Vomica. 20 ml. of 10 per cent hydrochloric acid is added to 20 ml. of ethanolic extract of nux vomica and heated on the water-bath until the ethanol is removed. The acid solution is filtered through a Whatman No. 1 filter paper and the residue is washed with dilute hydrochloric acid until the filtrate gave no precipitate with Mayer's reagent. The strychnine and brucine are precipitated as their reineckates and the free alkaloids are obtained in crystalline forms using the same procedure as described earlier. Strychnine is determined quantitatively first by treating the alkaloidal mixture with nitric acid and sodium nitrite followed by the extraction of strychnine from an alkaline solution with chloroform. The strychnine is back-extracted from the chloroform solution with acid and afterwards precipitated and determined as its reineckate. The results obtained are shown in Table 2.

Table 2. Determination of Strychnine in Nux Vomica

Volume of Nux Vomica extract used (ml.)	Amount of strychnine obtained (mgm.)	Percentage present
20	74·2	3·71
20	73·0	3·65
20	75·3	3·76
20 and 20 mgm. strychnine	93·7	3·68

I thank the chief chemist, Mr. Chia Chwee Leong, for his interest in this work and for his permission to publish these results.

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Action on Some Synthetic Peptides of a Protease from Rabbit Spleen

Some of the enzymes of bovine spleen have been studied in detail and classified as cathepsin *A*, *B* and *C* according to their specificity for certain synthetic peptides[1]. Examination of the action of rabbit spleen extracts on human serum albumin has shown the existence of a proteolytic activity having an optimal *p*H at 3·0–3·5 (ref. 2). It was shown that this activity was not due to an enzyme analogous with cathepsin *B* or *C* but could have been due to a cathepsin *A* (ref. 3). Recently we have demonstrated that this activity was due to a single protease which we have purified by chromatography on modified cellulose[4].

This communication deals with the action of the crude rabbit spleen extract and the purified protease on the synthetic peptides specific for the cathepsins *A*, *B* and *C* and carboxypeptidase of bovine spleen. The crude extract and purified protease were prepared from rabbit spleens as described previously[4]. The proteolytic activity of the two enzyme preparations was measured using human serum albumin as substrate[4]. The two preparations were employed in quantities such that their final concentration expressed in arbitrary proteolytic units was 35 U.P. in both cases. The final concentration of the substrates was 0·05 *M* in citrate buffer 0·1 *M*, *p*H 5·0. Cysteine, at a final concentration of 4 m*M*, was added to the different substrates with the exception of carbobenzoxy-L-glutamyl-L-tyrosine. The reaction media were incubated at 37° C. for 19 hr. except in the case of the hydrolysis of carbobenzoxy-L-glutamyl-L-tyrosine, by the crude extract, when the incubation time was 5 hr. The moles of carboxylic groups

Table 1. PERCENTAGE HYDROLYSIS OF THE DIFFERENT SYNTHETIC SUBSTRATES

Enzyme \ Substrate	CGluT	BAA	GPA	CGP
Crude extract, 4·2 mgm./ml.	38	60	116	45
Purified protease, 0·60 mgm./ml.	0	0	10	0

CGluT, Carbobenzoxy-L-glutamyl-L-tyrosine: substrate for cathepsin *A*; BAA, benzoyl-L-argininamide-hydrochloride: substrate for cathepsin *B*; GPA, glycyl-L-phenylalaninamide-acetate: substrate for cathepsin *C*; CGP, carbobenzoxyglycyl-L-phenylalanine: substrate for carboxypeptidase.

liberated during the reaction were measured by the method of Grassmann and Heyde[5] and the percentage of hydrolysis is given by the relation:

$$\frac{\text{Moles carboxylic groups liberated} \times 100}{\text{Moles substrate employed}}$$

As can be seen in Table 1 the four substrates were hydrolysed by the crude extract, thus demonstrating the existence in rabbit spleen of enzymatic activities analogous with those of cathepsin *A*, *B*, *C* and carboxypeptidase of bovine spleen.

With the purified protease there is no hydrolysis of the substrates specific for cathepsins *A*, *B* or carboxypeptidase. There is hydrolysis of the substrate for cathepsin *C*, but it is minimal and is most probably due to the presence of traces of cathepsin *C*. These results show that the protease from rabbit spleen is different from cathepsin *A*, *B*, *C* and carboxypeptidase.

The inactivity of the rabbit spleen protease toward the specific substrates of cathepsin *A*, *B* and *C* is an additional property in common between this enzyme and a protease recently isolated from bovine spleen by Press, Porter and Cebra[6].

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Immuno-Electrophoresis of Insulin-binding Antibodies

THE presence of insulin-binding antibodies in the sera of insulin-resistant diabetics has been shown by a large number of investigators[1]. It has also been shown by Berson *et al.*[2] and Kalant *et al.*[3] that using paper electrophoresis the binding was mainly in the γ-globulin, while using free-boundary electrophoresis the binding was mainly in the β-globulin. The disadvantage of using paper as a supporting medium is discussed in work now in course of publication[4]. In view of the differences of opinion as to which of the serum proteins is responsible for the binding of insulin it was decided to apply the techniques of immuno-electrophoresis with subsequent autoradiography to the study of diabetic sera.

The insulin was labelled with iodine-131, using iodine monochloride as the carrier by the technique described by McFarlane[5], and had a specific activity of 3·2 mc. per mgm. insulin. A 1 per cent solution of 'Ionagar' (Oxo, Ltd.) was prepared in a 0·5-μ concentration of barbitone buffer[6]. 15 ml. of this was poured on to glass plates 6 in. × 2½ in., and holes approximately 1·5 mm. diameter were cut at regular intervals along the centre line. Each hole was filled with a serum sample to which had been added about one-tenth of its volume of the tagged insulin solution. The serum samples therefore contained approximately 30 μgm. of tagged insulin per ml. The volume of sera in each hole was approximately 0·003 ml.

Immuno-electrophoretic controls of bromphenol blue and nitroaniline were used to mark the movement of the albumin and γ-globulin respectively, as suggested by Wunderly[7]. Electrical contact was made through Whatman No. 3 filter paper and a current of 15 m.amp. passed. Small strips of filter paper soaked in the appropriate anti-sera were placed between adjacent holes (Pasteur horse anti-human serum, and anti-*S*7 γ-globulin–Soothill). The patterns were allowed to develop for 1 week in a moist chamber. The glass plates were then taken out, covered with filter paper to remove the excess protein and salts, and allowed to dry at room temperature. The precipitation pattern was developed in 0·1 per cent nigrosin in 2 per cent acetic acid overnight and then washed in 2 per cent acetic acid to which a little 'Teepol' had been added to remove the excess stain. They were then put on end to dry.

Autoradiography was performed by placing the agar plate in contact with 'Ilfex' double-coated X-ray

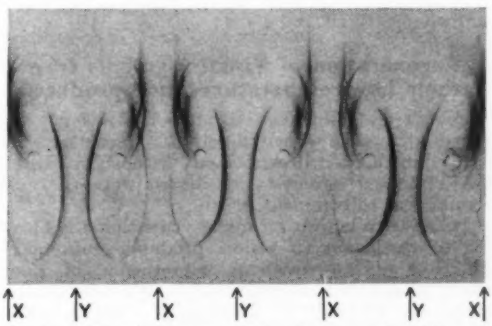

Fig. 1. Stained lines on agar plate. Arrows mark positions of strips of antisera. *X*, Pasteur horse anti-human serum; *Y*, anti-*S*7 γ-globulin

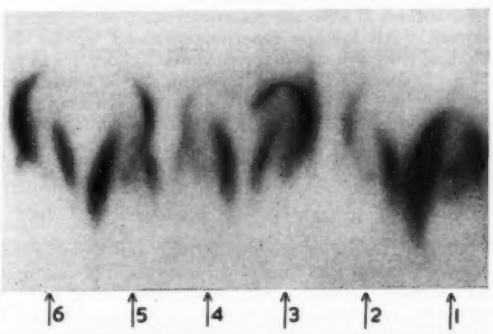

Fig. 2. Autoradiograph. Arrows mark positions of serum samples. Note serum sample 2 is the same serum as sample 1, but one-third of the concentration of active insulin used in all the other samples

film. Figs. 1 and 2 show the result of this technique. Fig. 1 is a photograph of a section of the stained and dried agar plate showing the results on 6 serum samples; Fig. 2 is the corresponding autoradiograph. In this case with an activity of approximately 0·04 μc. per sample an exposure of 12 days showed the result illustrated.

It will be seen that in the binding of the insulin two anti-bodies are responsible, one of which corresponds to the *S*7 γ-globulin and the other to a globulin corresponding in mobility to β-globulin.

It is hoped to report the results more fully shortly and to investigate the binding quantitatively by examination of the density distribution in the appropriate areas of the autoradiograph.

We wish to thank Dr. Soothill for a generous gift of immuno-electrophoretically pure anti-*S*7 γ-globulin, and Dr. John Bodman for much help with the immuno-electrophoresis.

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Regeneration of Visual Pigments from their Low-temperature Photoproducts

Under the above title Bridges has recently published an article which describes experiments said to be "in marked disagreement with the picture of the bleaching process" we have proposed[1]. We should like to comment briefly.

It has been known for many years that the bleaching of rhodopsin to yellow products—retinene, or indicator yellow—involves a sequence of photochemical and thermal reactions[2,3]. We have analysed these as follows[4–6]. Rhodopsin contains 11-*cis* retinene attached as chromophore to a protein, opsin. Light isomerizes the 11-*cis* chromophore to the all-*trans* configuration, yielding a first, highly coloured (red-to-violet) all-*trans* chromoprotein, called lumirhodopsin. All further reactions proceed in the dark, that is, they are thermal reactions. There are at least two of these. First, lumirhodopsin is transformed to another, orange-to-red, all-*trans* chromoprotein, metarhodopsin, by what is probably a configurational rearrangement of the opsin. Metarhodopsin is then hydrolysed to the yellow mixture of all-*trans* retinene and opsin (indicator yellow), and this is probably accompanied by further configurational changes of the opsin.

The synthesis of rhodopsin also can involve both photochemical and thermal reactions. If one mixes 11-*cis* retinene and opsin in the dark, the two join to form rhodopsin by a thermal process[7]. However, if one starts with the products of bleaching in solution —all-*trans* retinene and opsin—the retinene must be stereoisomerized before it can combine with opsin; and this can be done with light[8]. In this case, therefore, the formation of rhodopsin involves the photochemical stereoisomerization of retinene, followed by the thermal condensation of the appropriate isomer with opsin. Light can also regenerate visual pigments directly from the lumi- or meta-pigments by isomerizing the all-*trans* chromophore to the 11-*cis* configuration[4–6,9].

This is not a complete description of the bleaching and synthesis of visual pigments. The presence of further intermediates is implied by other observations, made under different experimental conditions in our own and other laboratories (cf. refs. 10, 11). However, we believe these to be the salient features: (1) photo-chemical stereoisomerization of the chromophore; (2) configurational re-arrangements of opsin; (3) combination of retinene and opsin by a Schiff base linkage, and its hydrolysis during bleaching. This scheme, first developed on the basis of observations with squid and cattle rhodopsins[4,9], has been confirmed with rhodopsins from five other species[5] (including two of the three used by Bridges), and with the chicken cone pigment, iodopsin[6].

In view of the number of reactions involved, it is evident that the analysis of the bleaching and regeneration of visual pigments is best performed under conditions such that the partial reactions take place one at a time, or as nearly so as possible. Bridges's experiments do not fulfil this condition, since they are performed in circumstances in which photochemical and thermal processes take place side by side. This is shown by the fact that in his experiments rhodopsin bleaches in the light to "orange products" and indicator yellow. The latter is always a product of thermal reactions, as has been shown repeatedly by lowering the temperature sufficiently during the irradiation (thus allowing only photochemical reactions to take place), and then warming in the dark[2–6,12].

It must be clear that this overlapping of reactions makes Bridges's attempt to analyse the bleaching and regeneration of rhodopsin into partial reactions exceedingly difficult. After close examination of his results, we see no present need to revise our interpretation of the mechanism of these reactions.

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Hubbard and Kropf's explanation of my results is based on the statement that the experiments were "performed in circumstances in which photochemical and thermal processes take place side by side". The implication here is that irradiation converted the parent visual pigment to an orange photoproduct, and that this photoproduct was unstable and decayed to indicator yellow. This is not so. It was explicitly stated that the orange photoproduct produced by irradiating frog rhodopsin at − 5° C. was thermally stable.

Thus irradiation for 1 hr. of frog rhodopsin at − 5° C. "generates a mixture of indicator yellow and a stable product with $\lambda_{max.}$ displaced about 20 mμ towards the blue. . . . At this temperature the photoproduct is stable (measurements at 20 mμ intervals from 380 to 620 mμ interlacing with return measurements from 610 to 390 mμ give a check of stability)"[1]. The whole spectrum was measured over the course of 1 hr., that is, the same period of time as for the bleaching operation. In the experiments carried out with conger eel and rhesus rhodopsins, the return measurements were actually slightly higher than the outward ones; far from decaying thermally to indicator yellow, the orange photoproduct was being regenerated from this substance.

It was shown experimentally that the rhesus photoproduct was photosensitive, yielding indicator yellow or retinene by a photochemical reaction. As was stated, the probable explanation for the presence of indicator yellow together with thermally stable orange photoproducts in the frog and conger eel experiments is that it was the product of photochemically bleaching these substances.

The use of light of long wave-length in the experiments reported (dominant wave-lengths of 550 and 580 mμ obtained from a Hilger–Barfit monochromator) makes it unlikely that regeneration from photoisomerized indicator yellow was involved in the thermal regeneration of rhodopsin. Even in the acid solutions used, indicator yellow has $\lambda_{max.}$ no higher than 440 mμ, and later experiments at *p*H 9·0, where indicator yellow has $\lambda_{max.}$ at 380 mμ, have yielded identical results.

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BIOPHYSICS

Plasticity of Wool

The quality of a wool is not determined solely by the dimensional characteristics of the fibres, such as fineness, length and crimp. Differences in the nature of the substance govern the selection of wools for particular purposes, and it has been shown that these differences can be measured by determining the plasticity of the fibres[1]. The measurements are made by observing the rate of creep of the fibres in distilled water at 22·2° C., usually under a constant load of 6 kgm./mm.^{2}. When log $(E - E_t)$ is graphed against t, where E_t is the percentage extension at time t (min.), and E is an arbitrarily chosen limiting extension, a linear relationship is obtained in the later stages of extension; the slope (k) of this line gives a simple measure of plasticity.

Because plasticity measurements are tedious, there have been several attempts to devise more rapid methods of characterizing the substance of different wools, and le Roux[2] has suggested that there is a simple direct relationship between the plasticity of a wool and its urea-bisulphite solubility[3]. This possibility had been examined in these laboratories before the publication of le Roux's note, and as the two sets of results were found to be contradictory, further experiments have since been carried out. Most of the wools in the new group were from South Africa, because le Roux's work was confined to such wools. Plasticity measurements were made under the conditions defined above, and the rigidly defined conditions of Dusenbury were adopted for determinations of urea-bisulphite solubility[4]. Both sets of results are given in Table 1.

Table 1

Wool	Plasticity (k)	Urea-bisulphite solubility (%)
64*s* Port Elizabeth Grassveld	91·4	55·0
60*s* Port Elizabeth Grassveld	86·2	41·2
66*s* East London	77·6	63·0
70*s* Cape Town	70·3	48·0
64*s* Cape Town	75·8	57·6
Cape Town	95·3	53·4
Queensland	42·6	47·3
70*s* Durban	92·6	60·3
64*s* Durban	73·1	56·2
60*s* Durban	112·8	57·9

These results, like the earlier ones, indicate that there is no simple relationship between the plasticity of wool and its urea-bisulphite solubility.

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Orientation of Cockchafers

A paper by Dr. F. Schneider published in 1957[1] suggested that cockchafers can orientate themselves in the magnetic field of the Earth. I have therefore carried out some experiments to detect the possible existence of permanent magnetic material in the body of cockchafers.

Two methods were used. First, I suspended parts of dead cockchafers at the end of a thin glass wire (length 1 metre, diameter 25μ) and in a magnetic field up to 20 gauss. It was found that no magnet could be present of such strength that in a field of 0·2 gauss it would experience a torque of more than 2×10^{-6} dyne cm. In the second method I used very sensitive magnetometers of the Royal Netherlands Meteorological Institute, De Bilt, which enabled me to make measurements on living animals. These measurements showed that in a field of 0·2 gauss the torque exerted on the supposed magnet must be smaller than 2×10^{-7} dyne cm.

If the supposed magnet were 0·01 cm. long, the mechanical force exerted by one pole of the magnet would have to be 2×10^{-5} dyne to produce a torque

of 2×10^{-7} dyne cm. If we suppose that the sense cells are stimulated by the pulling of a hair, then it is reasonable (assuming that elongation of a hair of about $1{\cdot}4 \times 10^{-10}$ cm. is detectable and Brownian motion limits the natural sensitivity of sense cells) to accept the threshold for the perceptible force as about 0·1 dyne (according to Hl. de Vries). Therefore I conclude that, if the cockchafer has permanent magnetic material at all, it cannot exert a mechanical force in the Earth's magnetic field greater than 2×10^{-5} dyne, which is much too small to be perceived by the sense organs of the cockchafer.

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PHYSIOLOGY

Hypoglycæmic Response to Leucine in a Leucine-sensitive Man

It has been reported previously[1,2] that certain individuals show a profound lowering of the blood glucose-level following ingestion of leucine or *iso*-valeric acid and that this is a familial trait.

We have studied the mechanism of this hypoglycæmic response in a fasting leucine-sensitive male aged 29 years (case 3[1]) using methods previously described to follow peripheral glucose metabolism in the forearm[3,4] and plasma insulin activity[5]. Following successive infusions into the brachial artery at rates of 7 mgm./min. for 20 min. of L-leucine and commercial *iso*valeric acid neutralized in saline, 10·5 gm. L-leucine was taken by mouth.

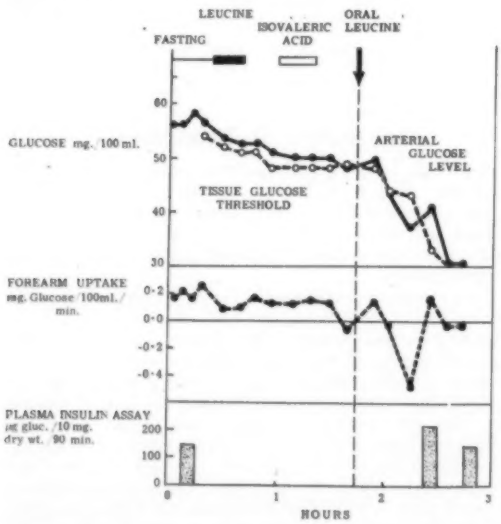

Fig. 1. Above, arterial blood glucose concentration ●---●, and glucose threshold ○——○; middle, glucose uptake by the tissues of the fore-arm (mgm. glucose removed from the arterial blood per 100 ml. tissue per min.); below, arterial plasma insulin activity (μgm. glucose taken up per 10 mgm. dry weight of rat hemi-diaphragm)

Fig. 1 shows the arterial blood glucose-level and plasma insulin activity, the glucose uptake and calculated glucose threshold of the fore-arm tissues. Neither leucine nor *iso*valeric acid, when continuously infused, affected the glucose uptake or glucose threshold of the fore-arm tissues. The oral dose of leucine, however, lowered the blood glucose-level and the tissue glucose threshold. The arterial plasma insulin activity increased significantly ($P<0{\cdot}05$) from 139 ± 20 to 212 ± 19 μgm. glucose/10 mgm. dry weight rat hemi-diaphragm/90 min. incubation, but this increase was only transient, results in accord with those published recently by Yalow and Berson[6].

The subsequent fall in the glucose threshold of the fore-arm tissues resembled that observed in normal individuals after injections of insulin[4]. It may be noted that the systemic fall of blood sugar was not accompanied by an increase of glucose uptake by the fore-arm tissues; this has also been observed in other subjects after intravenous insulin.

We conclude that: (1) it is unlikely that the peripheral tissues take up more glucose as a direct response to circulating leucine or *iso*valeric acid; (2) the hypoglycæmic effect of leucine in sensitive subjects is due to a transient rise in the plasma insulin activity.

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Effect of Strophanthin G on Oxidative Metabolism in Cardiac Muscle

Previous results published from this laboratory indicate that the increased mechanical efficiency (ratio of work output to energy input) apparent after the administration of non-toxic doses of cardiac glycosides to isolated spontaneously beating hearts is invariably associated with raised levels of oxidative metabolism[1,2]. Hajdu and Leonard[3] recently directed attention to the possibility that such an increased rate of oxygen consumption could be due to the increased mechanical work output associated with the positive inotropic response rather than to the fundamental metabolic action of the glycosides themselves.

An attempt has been made to investigate this possibility by comparing the effect of strophanthin *G* ('Ouabain' (Arnaud): Laboratoire Nativelle product) on the rate of oxygen uptake in actively beating hearts with that on hearts in systolic and diastolic arrest.

Experiments were carried out at 25·0° C. on hearts isolated from unselected summer toads (*Bufo*

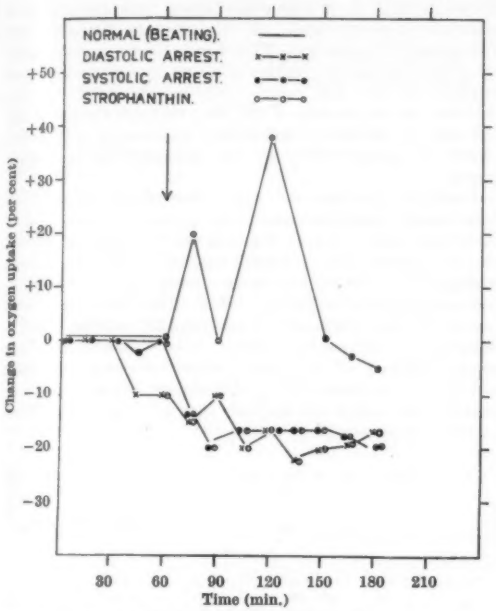

Fig. 1. Effect of strophanthin *G* (10 μgm./ml.) on the rate of oxygen consumption of actively beating hearts (mean 12 exp.) and hearts arrested in diastole (mean 6 exp.) and systole (mean 6 exp.). The drug was added at the arrow

marinus), using the perfusion apparatus and fluid previously described[4]. In six preparations isolated hearts were initially arrested in diastole by the addition of neutralized potassium chloride solution (minimal concentration). The rate of oxygen uptake was recorded at 15-min. intervals both during an initial control period of 1 hr. and throughout the 2 hr. following the addition of strophanthin *G* (final concentration 10 μgm./ml.). In a similar series hearts were arrested in systole by the administration of calcium chloride (neutralized), and the effect of strophanthin *G* on the oxygen consumption recorded as above. These results were compared with those previously recorded following the administration of strophanthin *G* at this same concentration to a series of actively beating hearts. The necessary control experiments were also carried out.

The results recorded are summarized in Fig. 1, from which it is evident that strophanthin *G*, at a concentration sufficient to produce a positive inotropic response[4], stimulated oxidative metabolism in actively beating hearts but failed to do so in those arrested either in diastole or systole. Similar observations were made in other experiments in which lanatoside *C* ('Cedilanide', Sandoz Ltd. product) was used (20 μgm./ml. final concentration). The rate of oxygen utilization in both the diastolic and systolic arrested control experiments gradually fell throughout the 3-hr. experimental period, while that of the control series of actively beating hearts remained relatively constant.

During the initial hour's perfusion, hearts arrested by potassium averaged an oxygen consumption of 1·59 ml./min./100 gm. wet weight (mean 12 exp.) compared with that of 4·233 ml./min./100 gm. wet weight (mean 10 exp.) recorded in the calcium arrested series.

These results demonstrate that, whereas the glycosides stimulate oxidative metabolism in actively beating hearts, they fail to do so in hearts which have been arrested. It may be argued that the increased rate of oxygen utilization recorded in the active series resulted from an effect of the glycosides on a specific stage during contraction or relaxation. Alternatively, as suggested by Hajdu and Leonard, the effect may be due primarily to the raised work output associated with the positive inotropic response in the active series. It seems unlikely that a gross metabolic effect could be involved.

In part these results explain the contradictory reports in the literature relating to the effect of non-toxic doses of glycosides on oxidative metabolism[3,5], since results from physiologically active preparations have been compared with those from inactive homogenate studies.

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Inhibition of Active Sodium Transport by Cholera Toxin

Active transport of sodium across cell membranes is now established as one of the fundamental physiological processes of most vertebrate cells. This process probably requires some sort of carrier molecule which binds sodium and which is coupled to a source of energy, the whole being designated for simplicity as a 'pump'. Failure of sodium transport may occur because of failure of energy supply, as, for example, in ischæmic skin and muscle after injury[1]. Certain drugs, such as cardiac glycosides, inhibit active transport by competing with sodium for the carrier[2]. One might also expect to find instances of failure or abnormalities of the pump itself, particularly in disease, but no examples of this are known.

Recent work[3] shows that in rat small intestine and colon, sodium is transported actively from lumen to blood, but that water transfer depends upon gradients of water activity and is a passive process. Net absorption of water, therefore, is dependent on active sodium absorption. If active sodium transport across the intestinal mucosa were decreased or abolished, one would predict the excretion of abnormally large amounts of water and sodium from the intestine, that is, diarrhœa.

This hypothesis could be tested by determining the effect on active sodium transport of crude extracts or toxins from micro-organisms which produce disease accompanied by diarrhœa. Cholera is an obvious example, and the preliminary results of such an investigation are reported here.

Experiments were carried out on abdominal skin and gastric mucosa from the European brown frog, *Rana temporaria*, and on skin from the American grass frog, *Rana pipiens*. The skin, or gastric mucosa, was fixed between two conical compartments filled with frog Ringer solution which was mixed by an air

pump. The potential difference across the tissue was measured, and in many experiments an external potential, applied through agar electrodes, was adjusted to maintain the potential difference at zero. The current produced by the untreated frog skin at zero potential (the 'short-circuit' current) has been shown by Ussing and Zerahn[4] to be equal to the net sodium flux. Therefore this current served in preliminary experiments to indicate changes in active sodium transport. In other experiments either the influx or efflux of sodium across the skin was measured with sodium-22, while the potential difference was held at zero, as described previously[5]. The flux in either direction could then be calculated from the relationship: net flux = influx − efflux.

All experiments reported here were performed with 'Cholera Filtrate Duphar' (N.V. Philips-Roxane, Amsterdam). The material was supplied freeze-dried in ampoules. The concentrations given are μgm. per ml. of Ringer solution in contact with the skin.

Fig. 1 shows a typical effect produced by the addition of cholera filtrate to both sides of the frog skin (725 μgm./ml.) Both short-circuit current and potential difference (measured by momentarily disconnecting the external electromotive force) decreased rapidly after addition of the cholera filtrate, a result characteristic of inhibition of active sodium transport. Fig. 1 also shows the effect of the same concentration of cholera filtrate on the potential difference and short-circuit current of the frog gastric mucosa. In this tissue only a transient increase in both occurred (possibly the effect of protein). Since the frog gastric mucosa does not actively transport sodium, this experiment indicates that the toxin is rather specific. In other experiments, similar to those shown in Fig. 1, it was established that the minimal effective concentration of the cholera filtrate used was about 60 μgm./ml. The same effect was produced by adding the toxin to the solution on the anatomical outside of the skin only. Adding the toxin to the solution on the inside of the skin did not decrease the current or potential difference, indicating a possible effect on permeability of the outward-facing membrane.

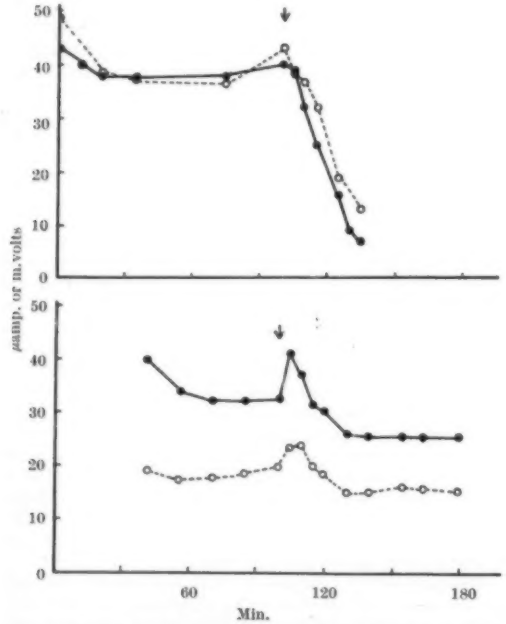

Fig. 1. The effect of cholera filtrate on the short-circuit current in μamp. per cm.² (solid lines) and the potential difference (broken lines) across the skin (above) and gastric mucosa (below) of the frog. The arrows indicate the time of addition of the cholera filtrate

Further experiments with skin from *R. pipiens* were made using sodium-22 as a tracer to prove that after addition of cholera toxin the decrease in short-circuit current was actually caused by decreased net sodium flux. Results from two representative experiments are given in Table 1. The influx of sodium was measured by addition of sodium-22 to the outside solution; net sodium flux was calculated from measurements of the short-circuit current; sodium outflux was calculated by difference. Cholera filtrate clearly decreased net sodium flux and sodium influx, that is, active sodium transport.

Table 1. Effect of Cholera Filtrate on Sodium Flux across the Frog Skin

	Time (min.)	Influx	Efflux	Net flux
Experiment 1				
Control	0–30	1·10	0·19	0·91
	30–60	1·10	0·25	0·85
Cholera filtrate	60–90	0·92	0·22	0·70
	90–120	0·44	0·12	0·32
Experiment 2				
Control	0–30	2·44	0·42	2·02
	30–60	1·99	0·27	1·72
Cholera filtrate	60–90	1·88	0·25	1·63
	90–120	1·11	0·05	1·06
	120–150	0·49	−0·09	0·58

Sodium fluxes are given in μmole/cm.²/hr. for successive 30-min. periods. Cholera filtrate (550 μgm./ml.) was added to the solution on the outside of the skin.

Only a few experiments have been performed to attempt to determine the nature of the component of cholera filtrate which inhibits active sodium transport. After dialysis through 'Cellophane' the active material is present in the dialysate. The activity is not measurably decreased by heating for 1 hr. at 80° C. Its properties therefore resemble those of the cholera endotoxin described by Burrows[6], and differ markedly from those of receptor destroying enzyme[7] (neuraminidase). Further experiments are in progress to determine the nature of the active material. The effect on sodium transport is consistent with our hypothesis for the diarrhœa of cholera.

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PATHOLOGY

Influence of Hyperthyroidism and of Thyroidectomy on Induced Mammary Cancer

It has been found[1] that the intragastric administration of optimal quantities of 3-methylcholanthrene induces mammary cancer with great rapidity in females of a susceptible strain of rats under standardized conditions; all the animals have invariably developed multiple mammary cancers within two months. Sex hormones are of critical significance[2] in the induction of mammary cancer by aromatic hydrocarbons. The effects of hyperthyroidism and of thyroidectomy were investigated in the present work.

Female rats of the Sprague–Dawley strain were received from a dealer at age 42 days, and certain groups were subjected to ovariectomy or thyroparathyroidectomy on the same day under ether anæsthesia. The thyroidectomized rats were supplied 1 per cent calcium lactate as drinking fluid thereafter. At age 63 days all the animals were fed 3-methylcholanthrene 10 mgm., dissolved in sesame oil (1 ml.) by gastric intubation, and this was repeated semi-weekly for 7 weeks. In addition, 3-methylcholanthrene was administered similarly to a group of intact rats for purposes of control, and to two groups receiving 1-thyroxine 0·5 mgm., or 1·0 mgm., respectively, beginning at age 63 days. 1-Thyroxine was freshly prepared daily and administered intraperitoneally 6 days each week for 7 weeks. The rats were examined daily for palpable mammary cancer and each tumour was confirmed histologically. The cytology of the vagina was studied frequently by preparing vaginal smears.

Among criteria for completeness of thyroidectomy were a profound depression of body-growth (Fig. 1) and the findings at necropsy of small adrenal glands (41·1 ± 8 mgm.) and a small spleen. The spleen was approximately one-third the size of that of intact rats; the adrenal glands of the intact control rats weighed 84·6 ± 10·8 mgm. Ten of the thyroidectomized rats had regular 5-day œstrous cycles, while two animals had irregular cycles. 1-Thyroxine (0·5 mgm.) did not modify the rate of body growth. Rats receiving 1-thyroxine (1 mgm.) grew at a normal rate for 3 weeks, but body growth was retarded significantly thereafter (Fig. 1).

All normal control female rats, and those receiving 1-thyroxine (0·5 mgm.), developed multiple mammary cancer (Table 1), which grew rapidly to a large size. Frequently the tumours attained a weight of 15 gm. by the ninetieth day of the experiment.

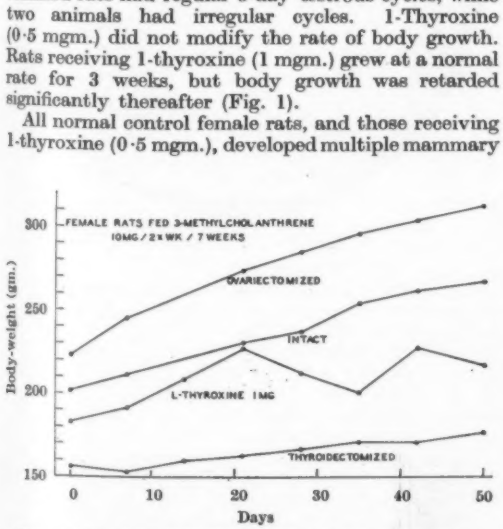

Fig. 1. Mean growth-rates of rats receiving 3-methylcholanthrene and other treatments

Table 1. Influence of Ovariectomy, Thyroidectomy and Thyroxine on Incidence of Mammary Cancer in Female Rats fed 3-Methylcholanthrene

	No. of rats	Rats with tumours	Appearance of palpable tumours (days)		
			Range	Median	Mean
Intact, controls	10	10	40–78	57	60·5 ± 12·1
Ovariectomized	10	3	66–127	—	—
Thyroidectomized	12	7	52–134	119	106 ± 28·5
1-Thyroxine (0·5 mgm.)	10	10	35–69	43	49·5 ± 11·6
1-Thyroxine (1 mgm.)	11	4	64–119	—	—

Ovariectomized or thyroidectomized rats and animals receiving 1-thyroxine (1 mgm.) had a significant decrease in the incidence of mammary cancer and a delay in their development. Only solitary cancers developed, and these remained small during observation for 6 months; the weight of the largest tumours was less than 100 mgm.

The decrease in incidence of mammary cancer in ovariectomized rats is certainly due to the deficiency of ovarian hormones. The decrease in the incidence of breast cancer in the thyroidectomized rats can be attributed to the influence of the consequent smaller calorie intake, which has been shown to have marked effects on the genesis of spontaneous and induced tumours[3,4]. In the case of rats receiving large amounts of 1-thyroxine some other mechanism is more likely. It was of interest that ovarian function was preserved in most of the thyroidectomized rats despite the depression of the rate of body-growth.

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Myxomatosis in a Californian Brush Rabbit (*Sylvilagus bachmani*)

Since myxomatosis was first reported near San Diego in 1930[1,2] there have been sporadic outbreaks of the disease in commercial rabbitries along the coast at least as far north as San Francisco. Sharp outbreaks of a disease highly lethal for European rabbits (*Oryctolagus cuniculus*) have been interspersed with long quiescent periods; a pattern which suggests that the disease is maintained elsewhere than in European rabbits used for commercial purposes. Although it has been suggested[2,3] that wild rabbits (*Sylvilagus* spp.) might be the reservoir, this has never been demonstrated.

Two simultaneous outbreaks of myxomatosis were reported in California during August 1959. One of these was at Palo Alto, 40 miles south of San Francisco, and the other was in commercial rabbitries between Vista and San Luis Rey, near San Diego.

The outbreak at Palo Alto was confined to one group of eight pet European rabbits, and a single rabbit in a group of seven on a neighbouring property. There are no commercial rabbitries in this semi-rural residential area, although there are several small colonies of pet rabbits scattered through the district. There appeared to be no connexion between this outbreak and the larger one in southern California.

Three brush rabbits (*Sylvilagus bachmani*) were trapped in the immediate vicinity of the affected colony at Palo Alto. One of these had a small and rather dry tumour on its left foreleg. This was removed aseptically and stored in sterile 50 per cent glycerol. Afterwards it was ground in a chilled mortar and suspended in calf serum saline containing antibiotics. An aliquot of 0·8 ml. of the approximately 10 per cent suspension was inoculated intradermally at several sites on the plucked flank of a domestic rabbit (*Oryctolagus cuniculus*). Small palpable nodules were detectable at the sites of inoculation on day 3, and these had merged into a flat plate-like tumour by day 5. The rabbit died on day 9 displaying typical but subdued symptoms of myxomatosis; a syndrome characteristic of the disease produced by the Californian prototype strain[3].

The sera of all three brush rabbits contained complement-fixing antibodies against myxoma antigen; the serum of the infected rabbit having a titre of 1/320. The antigen used in these tests was prepared with a strain of myxoma virus recently recovered during an outbreak in laboratory rabbits in Panama. This strain causes symptoms similar to the classical South American strains (for example, Brazil/Campinas/1949/1-Lausanne)[3]. The sera of sixteen brush rabbits trapped several miles from the outbreak area were negative.

This demonstration of the occurrence of infection with myxoma virus in wild *Sylvilagus bachmani* lends considerable support to the view that myxomatosis is enzootic in the wild rabbits of California, and it seems likely that the sporadic outbreaks of the disease in commercial rabbitries are due to transmission by mosquitoes from this reservoir. It will be recalled that this pattern is similar to that demonstrated by Aragao[4] for myxomatosis in South America, where the reservoir host is the tapeti (*Sylvilagus braziliensis*).

Observations made at both outbreak areas and results of current experiments on transmission and immunology of myxomatosis in brush rabbits are being prepared for publication elsewhere.

These investigations were aided by a grant from the Wool Research Committee of the Commonwealth of Australia.

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BACTERIOLOGY

Brucella Bacteriophage

Parnas *et al.*[1,2] obtained 14 samples of *Brucella* bacteriophage from old cultures of *Brucella abortus* and compared with these a *Brucella* phage originally isolated from manure in the U.S.S.R. The phages were described as being round, elementary bodies 60–80 mμ in diameter but with no tail. Cultures of *Br. abortus* were lysed by the phage, but cultures of *Br. suis*, *Br. melitensis* and a non-typical *Br. abortus* strains were not lysed. These observations have been confirmed by other workers[3,4].

A sample of the bacteriophage was received from Poland (through the courtesy of Dr. M. Kaplan, World Health Organization, Geneva). Originally, the phage was propagated by addition to young, actively growing cultures of *Br. abortus* strain 544 in Albimi broth but, later, a preparation with a much higher titre was prepared by the double layer method[5]. The phage was suspended in 0·033 *M* phosphate buffer containing 0·002 *M* magnesium, 0·00025 *M* calcium and 1/10,000 albumin[6]. Assay of this material on *Br. abortus* 544 gave a value of $2·8 \times 10^{10}$ plaque-forming particles/ml. Two kinds of plaques were found, one being 2·5 mm. in diameter and the others being 0·1–0·5 mm. in diameter (Fig. 1). Repeated subculture of single small and large plaques still gave rise to a mixture of small and large plaques.

Fig. 1. The different kinds of plaques on *Br. abortus* 544

For phage typing, *Brucella* cultures were grown on serum dextrose agar for 24–36 hr. and the growth suspended in buffered saline. A loopful of the suspension was cultured on well-dried, serum dextrose agar plates and 0·02 ml. of the phage preparation, at the routine test dilution of 10^{-3}, spotted on the culture. Readings were taken after 24 and 48 hr. of incubation. All cultures of *Br. abortus* (including those of type II Wilson) were lysed but none of 4 cultures of *suis*. However, of 33 cultures of *Br. melitensis* studied, 24 were lysed by the phage preparation. All the *melitensis* cultures that were lysed had been isolated in Great Britain—mostly from bovine milk samples.

In another experiment, the phage preparation was titrated using *Br. abortus* strain 544 and *Br. melitensis* strain *H* (isolated in Britain) as host cultures. The value against *abortus* was $2{\cdot}8 \times 10^{10}$ plaque-forming particles/ml. and against *melitensis* 9×10^{9} plaque-forming particles/ml. Therefore the *Brucella* phage is able to lyse not only *Br. abortus* but also some strains of *Br. melitensis* as well.

For electron microscopy, the phage was washed in 0·033 *M* phosphate buffer containing 0·00025 *M* calcium chloride and examined by the negative contrast method[7]. The phage particles were polyhedral, 65 mμ across, hexagonal in plan view and bore a wedge-shaped tail, 14 mμ long, attached to one corner (Fig. 2). They are indistinguishable from *coli* phage *T3* (Bradley, D. E., and Kay, D., unpublished work) and are believed to be the first example of this morphological type outside the *coli–typhoid* group of phages.

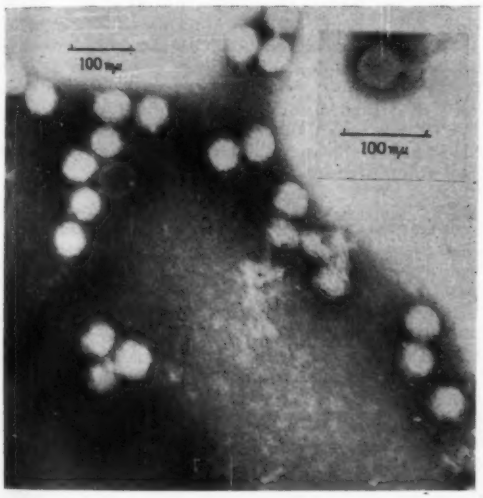

Fig. 2. *Brucella* bacteriophage. Negative contrast preparation with phosphotungstic acid

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Sensitivity to Dihydrostreptomycin of Enterobacteriaceae Strains on Media containing Ammonium

In the course of investigations of the fertility of some strains of Enterobacteriaceae, it was observed that *Citrobacter* (*E. freundii*) strains grew well on minimal medium plates supplemented with galactose 1 per cent and dihydrostreptomycin 100 μgm./ml. On the same medium, *E. coli* strains showed no growth. The *Citrobacter* strains were isolated during 1942–43 before the 'streptomycin era'. On complete medium with the same amount of dihydrostreptomycin, both *Citrobacter* and *E. coli* strains were completely inhibited. Even though an explanation of this phenomenon has not been reached as yet, a brief report on the results obtained so far will be given here.

The minimal medium was 'EMS', which contains ammonium sulphate as nitrogen source, magnesium sulphate, sodium chloride, dipotassium hydrogen phosphate, succinate, eosin methylene blue as indicator, and in this case galactose 1 per cent in addition. 'EMB', which was the complete medium used, contains peptone 1 per cent, dipotassium hydrogen phosphate, indicator and galactose 1 per cent. Plates were inoculated with one drop of a suspension (about 10^8 cells/ml.) in saline of a 20-hr. broth agar plate culture. The plates were read after 48 hr. at 37° C.

Table 1 shows the results of sensitivity tests on 'EMS' and 'EMB' medium supplemented with 100 μgm./ml. dihydrostreptomycin. A number of Enterobacteriaceae strains belonging to the biochemically defined genera *Salmonella*, *Arizona*, *Klebsiella* and *Cloaca* are included. Some *Shigella*, *Proteus*, *Providence*, *Hafnia*, *Morganella* and *Rettgerella* strains were also examined, but, as many of these grew only poorly or not at all on minimal medium, results with these strains are not recorded in Table 1. It appears that almost all *E. coli*, *Klebsiella* (and *Cloaca*) strains were sensitive to 100 μgm./ml. dihydrostreptomycin on both complete and minimal media. In contrast, all the *Salmonella* and practically all the *Citrobacter* strains were resistant to dihydrostreptomycin when tested on 'EMS' (minimal) medium, while the same strains were sensitive on 'EMB' (complete) medium. Some of the few *Arizona* strains examined were resistant and some were sensitive on 'EMS' streptomycin medium. In this connexion, it should be noted that the four *Arizona* strains that were streptomycin-resistant on 'EMS' medium were diphasic. None of the six streptomycin-sensitive strains was diphasic.

As mentioned above, the suspensions used for inoculation were made in distilled water from broth agar plates incubated at 37° C. for 20 hr. The results were the same when the inoculation suspen-

Table 1. Resistance of Enterobacteriaceae Strains to Dihydrostreptomycin on Minimal and Complete Media

	No. of strains	'EMB'		'EMS'	
		R	*S*	*R*	*S*
Salmonella	25	0	25	25	0
Citrobacter	31	0	31	26	5
Arizona	10	0	10	4	6
E. coli	205	0	205	0	205
Klebsiella	63	0	63	0	63
Cloaca	7	0	7	0	7

All media contain dihydrostreptomycin 100 μgm./ml. *R*, Growth; *S*, no growth. Strains which were streptomycin-resistant were excluded.

sions were prepared from minimal medium plates ('EMS' galactose).

Table 2 gives the results of sensitivity tests on 'EMS' and 'EMB' media supplemented with galactose and varying concentrations of dihydrostreptomycin, using strains selected at random from different genera of Enterobacteriaceae. It will be seen that the phenomenon described above for *Citrobacter* strains seems to be common for bacteria belonging to Enterobacteriaceae, that is, when high resistance to dihydrostreptomycin is found on minimal medium, a comparatively high, but in absolute figures much lower, resistance is found on complete medium. This also means that it is possible for all genera of Enterobacteriaceae to find a concentration of dihydrostreptomycin which will inhibit all growth on 'EMB' medium, while the same concentration on 'EMS' medium will have no visible effect on the growth. The level at which inhibition occurs varies between the different genera, and is exceptionally high for *Salmonella* and *Citrobacter*.

Table 2. Inhibitory Concentrations of Dihydrostreptomycin on Complete and Minimal Media

Genus	Strain	'EMB'	'EMS'
		Dihydrostreptomycin (μgm./ml.)	Dihydrostreptomycin (μgm./ml.)
Salmonella	4066*	128	8,192
Citrobacter	Ballerup 1	16	2,048
Arizona	Arizona origin	<2	32
E. coli	*K1*	<2	32
Klebsiella	*K20*	<2	16
Cloaca	8127/51	4	32

* *S. typhi murium.*

Tests were read after 48 hr. at 37° C. The figures indicate the plates with the lowest concentration of dihydrostreptomycin which showed no growth. The plates contained two-fold dilutions of dihydrostreptomycin from 2 μgm./ml. to 8,192 μgm./ml.

It should be mentioned briefly that the principle described could be used for differential diagnosis between genera of Enterobacteriaceae, provided that the results can be confirmed on a larger material. Furthermore, the minimal medium described seems to act as an excellent primary selective medium for *Salmonella* and *Citrobacter* strains.

In order to ascertain which factor(s) were responsible for the great difference in sensitivity on minimal medium and on complete medium, some new media were devised. Common to these was the following basic medium called *B*: sodium chloride 1 gm., magnesium sulphate 1 gm., dipotassium hydrogen sulphate 2 gm., glucose 10 gm., agar 20 gm., distilled water 1,000 ml. The following additions were made: *B*1 = *B* + peptone 'Orthana' 0·2 per cent, *B*2 = *B* + casamino-acid 'Difco' 0·2 per cent, *B*3 = *B* + ammonium sulphate 0·5 per cent, *B*4 = *B* + ammonium sulphate + peptone 'Orthana' 0·2 per cent, *B*5 = *B* + ammonium sulphate + casamino-acid 'Difco' 0·2 per cent.

These five media were supplemented with dihydrostreptomycin in two-fold dilutions from 0·07 μgm./ml. to 10,000 μgm./ml. One *coli* strain, the test strain for *O*-antigen 0100, and a *Salmonella typhi murium* strain *Br* 4066, were tested on these media. The plates were inoculated with suspensions from 20-hr. broth agar plates in amounts which would give about 500 colonies per plate on a complete medium without streptomycin. Table 3 shows the concentrations of dihydrostreptomycin which completely inhibit visible growth on the different media. It will be seen that the great difference in sensitivity found on *B*1 and *B*2 on one hand, and on *B*3, *B*4 and *B*5 on the other, can be explained by the presence of ammonium sulphate. Similar results were obtained when ammonium sulphate was substituted by ammonium chloride. It was further found that the use of 3–4-hr. broth cultures for inoculation did not change the results. The conclusion must be therefore that the presence of ammonium in the media will hinder the activity of dihydrostreptomycin to a very great extent, namely, 20–100 times more dihydrostreptomycin is necessary to inhibit growth on the minimal medium than on complete medium. This effect is not caused by differences in *p*H, as *p*H determination of the five different media just before pouring showed only variations from 6·8 to 7·1. In order to test whether dihydrostreptomycin inhibited the transport of amino-acids through the cell membrane, examination was made to see whether dihydrostreptomycin could hinder the growth of amino-acid-dependent mutants in media supplemented with the appropriate amino-acids. Three mutants of *E. coli* 0100, one histidine-dependent, one tryptophan-dependent and one leucine-dependent, were tested on *B*1 and *B*3 media supplemented by the amino-acids in question; dihydrostreptomycin concentration 2·35 μgm./ml. It was found that at least these mutants were able to utilize the amino-acids added to the media also when dihydrostreptomycin was present.

Table 3. Inhibitory Concentrations of Dihydrostreptomycin on Media with and without Ammonium Sulphate

	Media without ammonium sulphate		Media containing ammonium sulphate		
	*B*1	*B*2	*B*3	*B*4	*B*5
E. coli 0100	0·14	0·14	9·0	9·0	9·0
Salmonella 4066*	50	50	5,000	5,000	5,000

* *S. typhi murium.*

The experiment was carried out as plate dilution test. Figures indicate the lowest concentration of dihydrostreptomycin in μgm./ml. which will completely inhibit growth.

It would appear that dihydrostreptomycin interferes with the availability of nitrogen when this is present as amino-acids or more complex compounds. When the micro-organisms are able to utilize ammonia, the presence of ammonium salts will render the bacteria resistant to very high doses of dihydrostreptomycin.

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BIOLOGY

Cover on River Bottoms

In a previous communication[1], C_v was defined as the area of bottom covered by stones in a given area of stream bed. It was also suggested that this concept could be expressed as the ratio of the covered bottom to the sample area and written as a fraction varying from 0 to 1. The same term cannot, however, be used for both measure of the area and the fraction, and it is therefore proposed to restrict the term C_v to the prior definition so that C_v is expressed as an area. The term C_{vf} is proposed for the fraction covered

bottom/sample area and could be conveniently referred to as the 'cover fraction'.

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Tolerance to Maternal Skin Grafts in Rabbits induced by Hyaluronidase

Vascularized transplants are permanently successful only between identical twins or within a highly inbred strain. Even homografts from the mother to her litter are destroyed by the immunological process initiated by her transplant antigens[1]. However, tolerance for individual specific transplant antigens can be produced by the direct injection of donor cells into the fœtal or neo-natal animal[1]. These offspring accept grafts of normal tissue from the antigen donor. In contrast, uninjected control animals reject similar homologous transplants.

This report describes a biochemical method for the production of tolerance to maternal tissues by the intravenous injection of hyaluronidase into the pregnant rabbit. Presumably, the hyaluronidase alters permeability relationships so that the specific transplantation antigens pass from the pregnant rabbit to the litter.

Sixteen pregnant rabbits were given a single intravenous dose of 15–45 turbidity reducing units of hyaluronidase ('Infiltrase', Armour Laboratories, Chicago 11, Illinois). They were injected at 10–27 days after an observed coitus (Table 1). The gestation period in these outbred albino rabbits is 31 days. Nine litters from the injected mothers, a total of 44 progeny, were available for grafting when they were about 12 weeks old. Several rabbits failed to produce offspring, and some of the young died soon after birth.

Full-thickness skin grafts from the mother were applied to sites on the ears of the offspring. These transplants were about 3 cm. in diameter and were held in place with silk sutures. The grafts were observed daily. Rejection of a graft began with hyperæmia and œdema and was followed within 24 hr. by the formation of a hard dry scab. The initial hyperæmia was taken as the end-point of graft survival. All the control grafts were clearly devitalized by the fifth to the seventh day (Table 1). To eliminate the possibility that our sample of 10 controls might not adequately represent the total population we have assumed that only grafts remaining intact for more than 12 days demonstrate a significantly prolonged survival.

The experimental results (Table 1) indicate that intravenously injected hyaluronidase produces tolerance in the 11–19 day period of gestation. The progeny from animals injected on the tenth or after the twenty-second day of pregnancy failed to show a significant degree of tolerance to skin grafts. The best results thus far obtained are in the 11–15-day interval of fœtal development. Eight of the 15 young from pregnant mothers injected during the 11–15-day period of gestation showed significant prolongation of graft survival. One graft is still viable at 40 days and even shows some hair growth.

Of 44 experimental animals, 19 died during the course of these experiments. They are indicated by italicized numbers in the last column of Table 1. The cause of death has not been established. Only an occasional animal with stunted growth was observed.

Table 1. Survival of Skin Grafts from Mother to Offspring following the Intravenous Injection of Hyaluronidase into the Pregnant Rabbit

Exp. No.	Days of pregnancy at injection	Dose of hyaluronidase (turbidity reducing units)	Graft survival* (days)
1	—	none	6, 6, 5, 5
2	—	none	7, 7, 7, 7, 6, 6
3	10	30	12, 11, 9, 9, 8, 7
4	11	30	>40, 15, 14, 10, 8, *>6*, 6
5	14	30	*>12*, *>11*, 8
6	15	15	*>21*, *>18*, 9
7	15	30	14, 14
8	18	45	10, 7, 7, 7, 5
9	19	45	*>11*, *16*, >11, 11, *>9*, *>9*, *>9*, *>8*
10	22	45	*9*, *8*, 8, 8, *>6*, *6*, *6*, *4*, *4*
11	27	45	7

* Each figure is for a single member of the litter. The italicized values are for animals that died within 24 hr. of their last observation. The > sign indicates that the graft was in good condition at the time of death.

The pregnant mothers appeared unharmed by the low dose of hyaluronidase, 15–45 turbidity reducing units. The injection of 150–450 turbidity reducing units did not produce toxic effects in a few acute experiments.

Fig. 1 shows a microscopic section of a graft taken from experiment 6. This graft has good survival of all cell layers at 21 days. There is a mild infiltrate of lymphocytes.

Billingham, Brent and Medawar[1] tested 29 rabbits from four litters with grafts from their untreated

Fig. 1. Tissue section from a maternal skin graft 21 days after transplantation (Exp. 5, Table 1). All the cell layers are in good condition. A mild infiltrate of lymphocytes is present. (Hæmatoxylin and eosin, × 90)

mothers. None of the grafts survived for more than 12 days. These results, which we have confirmed in our control experiments (experiments 1 and 2), indicate that sufficient individual specific antigen to produce immunological tolerance normally does not pass from mother to fœtus. However, maternal tolerance has been produced in rats by Lengerova[2], who irradiated animals on the fifteenth day of their pregnancy. Despite many fœtal deaths, enough of the progeny survived to demonstrate immunological tolerance to their mother's tissues.

Porter[3] has recently demonstrated by direct injection that the twenty-second day of development in the fœtal rabbit represents the end of the period for production of tolerance. Our results also indicate that maternal tolerance terminates at this stage. The present preliminary results further show that maternal tolerance can be obtained by the intravenous injection of hyaluronidase as early as the eleventh day of fœtal development.

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A Gular Plate in *Albula vulpes* (L.)

THE Elopidae and Megalopidae are considered to be the most primitive families of teleosts chiefly for three reasons: (1) the presence of an unpaired gular plate; (2) the presence of a true ethmoidal commissure between the infra-orbital sensory lines of both sides[1]; (3) the conditions with regard to the skeleton of the caudal fin[2]. In addition it may be mentioned that the Elopidae still possess a bucco-hypophysial canal (in the parasphenoid)[3] and that in the Megalopidae there persists a conus arteriosus with two rows of valves. The latter primitive character is also encountered in *Albula vulpes* (L.), which has therefore also been regarded as a primitive teleost.

According to Ridewood[4] and statements in modern text-books (for example, Berg[5]) *Albula vulpes* lacks an unpaired gular plate. In order to examine this condition, I stained a head of the latter species (Grenada, West Indies, June 28, 1955) in alizarine. By removing the skin rostral to the branchiostegal rays I then found immediately below the corium a long, narrow, median bone plate (Fig. 1) which beyond question is a gular plate at a more advanced stage of reduction than the corresponding plate in *Elops* and *Megalops*. The detection of this plate naturally does not mean that *Albula vulpes* would have a closer relationship to the Elopidae and Megalopidae than what has so far been assumed, for as is clear from other facts, *Elops*, *Megalops*, and *Albula* all represent evolutional lineages of their own.

Fig. 1. *Albula vulpes* (L.). Head viewed from the ventral side. (2/3 nat. size)

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Variations in the Serum Specificities of Higher Primates detected by Two-Dimensional Starch-Gel Electrophoresis

THE proteins of human serum subjected to two-dimensional zone electrophoresis, a combination of filter-paper and starch-gel electrophoresis, can be resolved into at least 21 distinct components[1,2]. Some of these proteins, for example, haptoglobin and transferrin, were found to be made up of components which exhibit genetically controlled variations in the human population[1–3]. To enlarge our view of the evolutionary process underlying such variability in serum specificities, we began a comparative study of the distribution of protein components in the sera of the Primates. So far, sera from 13 chimpanzees, 19 cynomolgus monkeys, 17 rhesus monkeys, and several other macaques in addition to human sera have been analysed by two-dimensional zone electrophoresis in a discontinuous system of buffers[4,5]. This communication reports on the results gathered up to now which enable us to distinguish the four primate groups and which emphasize a taxonomic gradation in the differences among the sera examined.

Examples of the typical starch-gel patterns of human, chimpanzee, rhesus and cynomolgus sera are shown in Fig. 1. It can be noted that each pattern has its own characteristic constellation of components. The human and chimpanzee patterns show remarkable similarities in respect to prealbumin, albumin, α-1 globulins (especially the presence of orosomucoid in the chimpanzees) and the fast moving α-2 globulins.

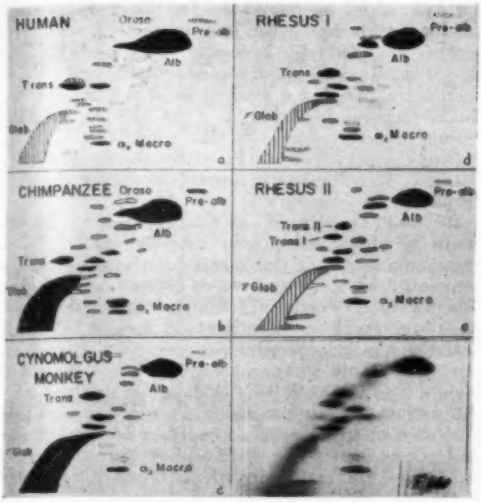

Fig. 1. Two-dimensional starch-gel electrophoresis of higher primate sera. The typical results in the four primate groups are represented diagrammatically. In addition, a photograph of the serum pattern of the rhesus monkey with the two transferrins is also presented

However, the two Hominoidea differ in the following way: the transferrin of the chimpanzees migrated more slowly on filter paper than the transferrin of the humans, and the α-2 macroglobulin (*S*α-2) of the chimpanzees migrated more rapidly than the human protein. The amount of gamma globulin in the chimpanzees always exceeded the amount found in humans.

The starch-gel patterns of the macaques (cynomolgus and rhesus) could be distinguished from those of the humans and chimpanzees in several ways. For example, the prealbumin could barely be detected and the amount of albumin was not as large. Perhaps the clearest diagnostic feature of the macaque patterns was the absence of a fast-moving α-1 globulin of orosomucoid mobility, and the presence of a new component which has the filter paper mobility of a γ-1 globulin but in starch-gel migrates faster than gamma globulin and slower than transferrin.

The closer taxonomic relationship of the cynomolgus and rhesus monkeys to each other than to any of the other primates is clearly reflected in the starch-gel patterns. Whereas a number of features in the patterns distinguish the macaques from the higher anthropoids (as we have indicated above), very few features distinguish the sera of *Macaca* (cynomolgus) *irus* from *Macaca* (rhesus) *mulatta*. In respect to the intrageneric differences, we observed that the cynomolgus sera always had a much larger quantity of gamma globulin.

Furthermore, in our limited sampling, the rhesus monkeys showed more intraspecific variation in serum pattern than the cynomolgus monkeys. An example of this is presented in Fig. 1*e*, in which a second transferrin can be seen to be directly ahead of the first by both the filter paper and starch-gel separations. This result confirms the observation of Bangham and Tee, reported by Allison[6], of a β-globulin variation among rhesus monkeys. In our work 10 of the 17 rhesus sera showed the additional component in contrast to the 19 cynomolgus sera which only showed the one transferrin. It is of interest that the rhesus monkey occupies a broader ecological habitat than the cynomolgus, and because of this, one is tempted to speculate that between the two species the rhesus will show more examples of polymorphism. Obviously, extensive surveys in natural populations will have to be conducted before conclusions of this type in respect to the Primates can be arrived at.

The identity of the various components was established by immunochemical methods, of which immunoelectrophoresis[5] was particularly suitable. For this purpose, we employed rabbit antisera (supplied by the Behringwerke, Marburg, Germany) to human γ-globulin, transferrin, α-2 macroglobulin, albumin, and prealbumin[7]. We also employed chicken antisera against the same proteins and against orosomucoid[8]. The primate sera belonged to the collection gathered for a previous study[9]. It is again a pleasure to thank Drs. G. D. Brigham, Sidney Gaines, Albert B. Sabin, and George B. Rabb for providing these samples. The generosity of Prof. H. E. Schultze in having portions of his highly specific rabbit antisera sent to us is also appreciated. This research was supported by the National Institutes of Health, National Multiple Sclerosis Society (U.S.A.), and Children's Fund of Michigan.

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Metabolism of D-Xylose by Moulds

The filamentous fungi have been called ubiquitous and omnivorous, and certainly they are widely distributed in Nature where they play a most important part in the decomposition of complex organic matter and as pathogens for many plants. Since much of the plant-derived organic matter in Nature contains a large amount of pentoses, mostly in the form of pentosans, a study of the pentose metabolism of the fungi which decompose this material is of interest not only because it is a basic phenomenon, but also, as we have found, because it is somewhat unique.

Early work on the fermentation of D-xylose-1-^{14}C by the resting cells of *Fusarium lini* Bolley suggested a dissimilation similar to that in *Lactobacillus pentosus*[1]; namely, the conversion of the D-xylose to equimolar quantities of carbon dioxide, ethanol and acetic acid presumably following a split of the molecule into a C_2 and a C_3 fragment. Another aspect of pentose metabolism in filamentous fungi is the formation of mycelial glucosan from pentoses labelled with carbon-14 by an unknown pathway in *Aspergillus niger*[2]. Indeed, there is but little information

on the enzymatic sequences and intermediates of pentose metabolism in any of the fungi. In contrast, recent intensive studies on the metabolism of pentoses by various bacteria have revealed two common features in the sequence of intermediates. Metabolism is initiated with the conversion, by the appropriate isomerase, of the aldopentose to the corresponding ketopentose; specifically D-xylose and L-arabinose are converted to D-xylulose and L-ribulose respectively[9–11]. The second feature common to the utilization of the pentoses is the conversion of the ketopentose to the important intermediate D-xylulose-5-phosphate. This is accomplished by the phosphorylation of the ketopentose followed by epimerization to D-xylulose-5-phosphate[12,13]. The latter intermediate is further metabolized by well-established pathways.

During the course of work in this laboratory on D-xylose metabolism by cell-free extracts of *Penicillium chrysogenum* an enzymatic sequence was found for the interconversion of pentoses which is quite different from that in the bacteria. Instead of being converted to the corresponding ketopentose, D-xylose is reduced rapidly to D-xylitol by a triphosphopyridine nucleotide-linked reductase, followed by oxidation of the D-xylitol to D-xylulose or L-xylulose with a diphosphopyridine-linked dehydrogenase. Whether D- or L-xylulose is formed depends upon whether the C_2 or C_4 carbon is oxidized, since xylitol is symmetrical[14,15]. When the mould is grown on L-arabinose a similar enzymatic mechanism converts this pentose to L-ribulose and L-xylulose with the formation of L-arabitol as an intermediate[16].

To determine how general this metabolic pattern is among micro-organisms a study was made on fourteen species of moulds and yeasts selected from nine genera according to their ability to grow on D-xylose as the only carbon source and their common occurrence in Nature. The cultures were transferred twice on a xylose-mineral salts-agar slant and spores from the second slant were inoculated into the same medium minus the agar[15]. This synthetic medium was not the best for all the fungi; however, sufficient growth occurred after 72 hr. on a rotary shaker so that the cells could be harvested for the preparation of cell-free extracts. The enzyme preparation from yeasts was made by breaking the cells with small glass beads in a 10 kc./s. sonic oscillator and from the moulds by grinding a washed sand–cell mixture in a chilled mortar. Table 1 gives the experimental details and the results of this general survey. The xylitol and xylulose produced by the fungi listed in Table 1 were further identified by paper chromatography.

It should be pointed out that no attempt has been made to obtain maximum activity for any of the organisms and that the specific activities cannot be associated with the amount of growth on D-xylose in this particular medium. All the *Aspergillus* species grew luxuriantly on D-xylose and showed low specific activity for xylose reductase, whereas *Myrothecium verrucaria* grew comparatively less and showed the highest specific activity. A possible explanation of the variation in xylose reductase activity is that the glucose-6-phosphate is susceptible to phosphatases and consequently the triphosphopyridine nucleotide reducing system is destroyed. This was quite apparent with the *Neurospora crassa* experiments where considerable free glucose was evident on the paper chromatograms that were used to separate D-xylose and xylitol. With the exception of *Neurospora crassa* and *Myrothecium verrucaria*, the rate of reduced diphosphopyridine nucleotide oxidation in a mixture of D- and L-xylulose was equal to the sum of the rates on each isomer alone, indicating the stereospecificity of two enzymes; this may not be true with the exceptions mentioned.

The fourteen fungi listed in Table 1 as possessing this unique method of D-xylose metabolism were the ones in the twenty which were tested that grew on D-xylose. *Escherichia coli*, *Aerobacter aerogenes*, *Bacillus subtilis*, *Bacillus cereus*, and *Pseudomonas aeruginosa* grew well in a medium with D-xylose as the only source of carbon, but their cell-free extracts were devoid of the reductase. It seems that this unique pathway for the metabolism of aldopentoses is confined to filamentous fungi and yeasts, thus

Table 1. Specific Activities of Xylose Reductase and Xylitol Dehydrogenase in Certain Fungi grown on D-Xylose*

Fungi examined	Xylose reductase†	Xylitol dehydrogenase‡	Reduced diphosphopyridine nucleotide oxidation§		
			D-Xylulose	L-Xylulose	D-Xylulose and L-xylulose
Penicillium chrysogenum	2·32	0·217	1·61	1·61	3·23
Penicillium citrinum	2·33	0·261	4·58	2·68	7·20
Penicillium expansum	2·34	0·136	5·68	2·84	8·50
Aspergillus niger	0·65	0·100	1·98	2·05	3·98
Aspergillus oryzae	0·33	1·41	2·61	2·13	4·65
Aspergillus fumigatus	0·32	0·084	2·99	1·21	4·20
Aspergillus ochraceus	0·23	0·065	0·82	0·61	1·39
Rhizopus nigricans	1·53	0·088	3·08	1·63	4·61
Gliocladium roseum	1·71	0·198	3·39	1·61	5·16
Byssochlamys fulva	1·01	0·081	7·75	15·80	26·00
Myrothecium verrucaria	2·89	0·256	11·30	7·10	14·15
Neurospora crassa	0·40	0·079	21·00	7·26	22·60
Rhodotorula glutinis	1·02	0·071	13·70	7·43	20·93
Torulopsis utilis	1·39	0·112	22·10	18·55	40·15

* All the activities are corrected for endogenous reactions.

† The assay system contained 30 μmoles glucose-6-phosphate, 0·2 mgm. glucose-6-phosphate dehydrogenase, 1 μmole triphosphopyridine nucleotide, 10 μmoles magnesium chloride, 30 μmoles D-xylose and 10 mgm. protein in a total volume of 2·0 ml. of 0·05 *M* phosphate buffer at *p*H 7·2. Incubation was at 30° for 40 min. Aliquots were analysed simultaneously for xylose and xylitol by the orcinol and periodate oxidation procedures respectively after the reaction was stopped by zinc barium precipitation. The specific activity is expressed as μmoles xylose reduced per mgm. protein.

‡ The assay system contained 40 μmoles xylitol, 20 μmoles diphosphopyridine nucleotide, 10 μmoles magnesium chloride and 10 mgm. protein in a total volume of 2·0 ml. of 0·1 *M tris* buffer at *p*H 8·5. Incubation was at 30° for 1 hr. Aliquots were analysed for ketopentoses by the cysteine–carbazole colour test. The specific activity is expressed as μmoles ketopentose formed per mgm. protein.

§ The assays were made spectrophotometrically at 340 mμ. The system contained 0·16 μmole reduced diphosphopyridine nucleotide, 15 μmoles of either ketopentose or both and enough enzyme protein to give a 0·1 *O.D.* change per min., in a total volume of 0·1 ml. of 0·1 *M* phosphate buffer at *p*H 7·5. The readings were taken after a reaction time of 2 min. The specific activity is expressed as μmoles × 10^3 reduced diphosphopyridine nucleotide oxidized per min. per mgm. protein.

differentiating them biochemically from bacterial and animal cells.

A portion of this information was presented at a meeting of the Society of American Bacteriologists during May 1–5. The work was supported by grant E-1201 from the National Institutes of Health.

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Structure of *Acrocomia* Fruit

Of the palm kernels utilized as feeding materials, those of the coconut (*Cocos nucifera* L.) and palm nut (*Elaeis guineensis* L.) are best known. The differences in structure of the endosperm cells probably provide the most reliable means of distinguishing between the two species in meals or cakes. The endosperm cells of the coconut are more elongated than those of the palm nut and their walls are uniformly thickened, as opposed to the pitted walls of *Elaeis*[1].

Occasionally, commercial samples are obtained under the description of Paraguayan palm. The botanical name for this plant has been given as *Acrocomia totai*. This palm is an important oil plant in Paraguay[2] but no descriptions have been found of the histology of the fruit. Studies of the histology of fruits and seeds can be important in plant taxonomy and are also valuable in the identification of commercial plant products.

An investigation has been carried out into the histological structure of the commercial samples of Paraguayan palm with the view of establishing some

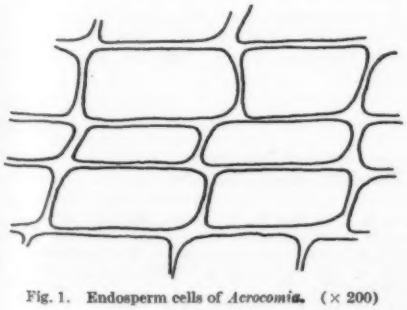

Fig. 1. Endosperm cells of *Acrocomia*. (× 200)

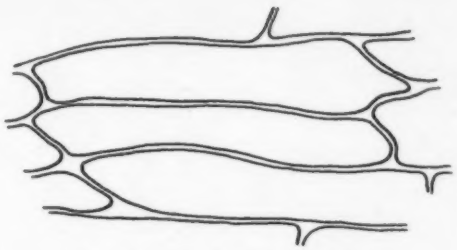

Fig. 2. Endosperm cells of *Cocos*. (× 200)

means of distinguishing between them and samples of coconut and palm nut. To augment this material, fruits of *Acrocomia sclerocarpa* Mart. have been obtained from Brazil. This species is very closely related to *totai*[2].

It has been found that, as in the case of *Cocos* and *Elaeis*, a consideration of the structure of the endosperm is most useful for the purposes of identification. Fig. 1 illustrates some typical endosperm cells of *Acrocomia* as they appear in a cleared preparation. The cells are brick-shaped, and their walls show no obvious pits. The absence of pits immediately distinguishes *Acrocomia* from *Elaeis*. In comparing the endosperm with that of *Cocos*, the shape of the cells provides the main distinguishing feature. Fig. 2 illustrates some typical *Cocos* endosperm cells. These cells are much more elongated than those of *Acrocomia*, a feature shown equally well in a longitudinal section of endosperm or a cleared preparation of a cake or meal.

It is intended to publish the details of this investigation elsewhere. I am indebted to Salamon and Seaber (consulting analytical chemists) for the supply of certain material used in this work.

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Changes in Sex in the Hop caused by Plant Growth Substances

DURING an experiment to test the effects of certain systemic chemicals on the hop plant (*Humulus lupulus* L.) the production of male flowers by a genetically female plant was noticed. This behaviour may occur naturally in some varieties; but the phenomenon has not been reported in the variety Fuggles which was used in the present experiments. These were designed originally to determine whether treatment with low concentrations of certain systemic chemicals would lead to changes in the chemical composition of the plant. For this purpose plants were sprayed with aqueous solutions of a number of phenoxy and phenylthio acids which, although similar in structure to plant growth substances, were either inactive or only weakly active[1].

Three levels of treatment were given, the plants being sprayed to run-off with 500 p.p.m. solutions of each of the acids as their sodium salts either once, twice or three times, with intervals of several days between sprayings. When flower parts appeared it was observed that male flowers had only developed

Fig. 1. Production of male organs on a genetically female plant following the application of α (2-chlorophenylthio)propionic acid

on those plants which had been sprayed with solutions of α (2-chlorophenylthio)propionic acid; three plants showed this effect, not all of which had received the maximum spray treatment.

In the following year an experiment was designed specifically to test whether the stage in growth of the plant at which the spray was applied was an important factor in relation to male flower development. The spraying was carried out as before with a 500 p.p.m. solution, and in a row of about 30 plants a different plant was sprayed at two-day intervals, beginning on July 2 and continuing until the beginning of August. The plants not sprayed were left as controls.

When the flowers developed it was seen that a large number of male flowers had developed on those plants sprayed on July 2 and 4; there were fewer on the plant sprayed on July 6, and practically none on those which were sprayed afterwards. The plants sprayed on July 8 and 10 showed slight deformities of the cones, but those plants which had received the later sprayings showed no abnormal effects and could not be distinguished from the controls.

Heslop-Harrison, in reviews of conditions governing sex expression in flowering plants[2,3], describes changes of sex resulting from treatments with the plant growth substance 2:4-dichlorophenoxyacetic acid or with the plant growth hormone indoleacetic acid. It seems possible that the weak growth properties of α (2-chlorophenylthio)propionic acid may operate in inducing the responses reported here. In this connexion, however, it is important to note that in all cases reported previously, growth substance treatment caused a change from male to female flowering whereas the changes reported here have been from female to male.

It is of interest that the experiments quoted in the literature where sex changes appeared were carried out with short-day flowering plants; the suggestion has been made, however, that long-day plants may not follow the same pattern[5], it having been observed that spinach tended to produce male flowers under short-day conditions.

Heslop-Harrison has suggested that photoperiodism may be related to natural auxin concentration; if this is the case with spinach, then either auxin concentration is lowered under short-day conditions or the initiation of male flowers arises from high auxin-levels. While the former possibility is unlikely, the latter supposition is difficult to correlate with the findings that with other plants, application of growth substances leads to the formation of female flowers.

The present experimental results with hop plants throw light on this problem if it be assumed that, as long-day plants, the hop and spinach behave similarly with respect to both day-length and applications of growth substances. A distinction may then be drawn between a long-day and a short-day flowering plant; in the former, high auxin-levels might promote the growth of staminate structures whereas in the latter, high auxin levels might promote pistillate structures.

I wish to thank Prof. R. L. Wain for his guidance and encouragement, the Hop Research Department for facilities, and Mr. R. A. Neve for his valuable help.

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STATISTICS

Structural Relations in Component Analysis

In a recent communication, Hey and Hey[1] have described Berkson's postulate of the 'controlled variable' as unjustified and his mode of analysis as invalid. After correspondence with Hey and Hey, we feel that it is their criticisms that are not valid; had they been, they would have applied equally to the whole of regression theory. In particular, they do not seem to attach sufficient weight to the need for a careful specification of the reference distribution with which the observations are to be compared.

In our communication[2] to which Hey and Hey refer, we included Berkson's method in a list of several which can be appropriate in various circumstances; we should perhaps have stated more clearly that differing experimental conditions necessitate differing analyses, and that no one method (not even that of least squares) has overall applicability. We did point out that in some versions of the structural relations problem, the method of least squares has been shown to lead to inconsistent estimates; so that in all but the simplest cases consistency and efficiency have to be justified *de novo*.

The problem considered by Hey and Hey[1] of fitting ν linear relations to a set of n-dimensional observations all subject to error is essentially equivalent to component analysis, for which see, for example, Girshick[3] or Kendall[4]; and has been treated explicitly in econometric contexts, for example, by Tintner[5].

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FORTHCOMING EVENTS

*(Meeting marked with an asterisk * is open to the public)*

Monday, October 3

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. W. B. Emery: "The Antiquities of Nubia and the High Dam".

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. G. L. S. Shackle: "Time, Nature, and Decision".

SOCIETY OF ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. W. A. Crago: "Some Notes on Hovercraft".

Wednesday, October 5

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. J. S. Elliott and Mr. E. D. Edwards: "Synthetic Lubricants for Gas Turbines".

INSTITUTE OF INFORMATION SCIENTISTS (at Swedenborg Hall, Barter Street, Bloomsbury Way, London, W.C.1), at 6.15 p.m.—Dr. G. Malcolm Dyson: "New Research at Chemical Abstracts".*

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Scientific Papers.

Thursday, October 6

ROYAL INSTITUTION OF NAVAL ARCHITECTS (at 10 Upper Belgrave Street, London, S.W.1), at 5.15 p.m.—Mr. R. N. Newton and Mr. H. P. Rader: "Performance Data of Propellers for High Speed Craft".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Sir Hamish D. Maclaren, K.B.E., C.B.: Inaugural Address as President.

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Prof. A. A. Eddy: "The Chemistry of the Cell Surface".

Friday, October 7

BRITISH MYCOLOGICAL SOCIETY (in the Large Physics Lecture Theatre, Birkbeck College, Malet Street, London, W.C.1), at 10.45 a.m.—Meeting on "The Cereal Rusts".

INSTITUTION OF ELECTRICAL ENGINEERS, MEDICAL ELECTRONICS DISCUSSION GROUP (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Nerve Endings" opened by Dr. O. C. J. Lippold and Dr. D. R. Wilkie.

SOCIETY OF DYERS AND COLOURISTS (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 6 p.m.—Prof. C. F. Carter: "Science in Industry".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. D. H. Hey, F.R.S.: "Some Recent Developments in Heterocyclic Chemistry".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT EXPERIMENTAL OFFICER (with at least G.C.E. in five subjects, including two (science or mathematics) at "A" level, or equivalent, and laboratory experience in chemistry or biochemistry) IN THE BIOCHEMISTRY DEPARTMENT—The Secretary, Institute of Animal Physiology, Babraham, Cambridge (October 8).

LECTURER OR SENIOR LECTURER (with qualifications in educational psychology and/or history and philosophy of education, and teaching experience) IN EDUCATION at the University of Queensland, Australia—The Secretary Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, October 8).

SENIOR LECTURER IN PURE MATHEMATICS—The Registrar, The University, Leicester (October 8).

ASSISTANT LECTURER (with special qualifications in physical chemistry) IN CHEMISTRY—The Registrar, The University, Manchester (October 15).

ASSISTANT TECHNICAL OFFICER (with G.C.E. in at least five subjects, two of which at advanced level (preferably including biology), or equivalent Scottish or Northern Irish qualification), for work in biological electronics and construction of apparatus for physiological research—Head of the Department of Zoology and Comparative Physiology, The University, Birmingham 15 (October 15).

PRINCIPAL LECTURER IN PHARMACEUTICS—The Principal, College of Technology, Belfast (October 21).

CHAIR OF GEOLOGY in the University of Malaya, Kuala Lumpur—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Malaya and London, October 22).

LECTURER IN MATHEMATICS, to take the subject to pass degree standard—The Principal, Bede College, Durham (October 22).

LECTURERS OR ASSISTANT LECTURERS (3) IN THE DEPARTMENT OF MATHEMATICS, University of Malaya, Kuala Lumpur—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Malaya and London, October 22).

SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER (with a first- or second-class honours degree in chemistry or equivalent, and well acquainted with the application of modern techniques to the analysis of foods and feeding-stuffs including estimation of amino-acids, and preferably previous experience with tropical foods) at the Tropical Products Institute (D.S.I.R.), London, W.C.1, to take charge of the analytical work in the Food Section—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting Ref. F.653/0A (October 29).

SCIENTIFIC OFFICER (with a first- or second-class honours degree with physical or inorganic chemistry as the principal subject) IN THE DEPARTMENT OF PEDOLOGY, for work involving investigations upon soil formational processes with a physicochemical bias—The Secretary, Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (October 29).

SENIOR SCIENTIFIC OFFICER (with a first- or second-class honours degree in a science, preferably chemistry, or equivalent, at least three years appropriate postgraduate experience, a wide interest in general scientific matters, and an aptitude for administration and dealing with people) in the Information Division, D.S.I.R., H.Q., London, to assist a survey team preparing detailed reports on selected scientific subjects and to undertake comprehensive surveys of scientific and technological problems in co-operation with representative and national and international organizations—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting Ref. F.666/0A (October 29).

ASSISTANT LECTURER OR LECTURER IN THE DEPARTMENT OF VETERINARY PATHOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/T (October 31).

LECTURER IN PHYSICS at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, October 31).

ASSISTANT LIBRARIAN (graduate with a professional qualification) at the University College of Sierra Leone (Fourah Bay College)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 10).

LECTURER OR ASSISTANT LECTURER IN GEOLOGY at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, November 10).

ASSOCIATE PROFESSOR OF STATISTICS IN THE FACULTY OF AGRICULTURAL ECONOMICS, University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, November 11).

PHYSICISTS AND RESEARCH ENGINEERS (with a first- or second-class honours degree or corporate member of a senior engineering institution, or with equivalent qualifications), for research work in the Engineering Studies Branch of the Reactor Development Division; field of research covers a very wide range of basic engineering topics, with particular emphasis on methods of extracting heat from power-producing nuclear reactors, cooled by gases, liquids and two-phased coolants—The Personnel Branch (W.340/34), U.K.A.E.A., A.E.E., Winfrith, Dorchester, Dorset (November 13).

MAY READER IN MEDICINE—The Registrar, University Registry, Oxford (December 2).

READER IN SOIL SCIENCE—The Registrar, University Registry, Oxford (December 2).

ASSISTANT EDITOR (with a physics degree and a knowledge of physics literature) for Section A (Physics) of the monthly publication *Science Abstracts*—The Secretary, Institution of Electrical Engineers, Savoy Place, London, W.C.2.

ASSISTANT LECTURER OR LECTURER (with an interest in vertebrate physiology) IN BIOLOGY—The Principal, Derby Training College, Uttoxeter New Road, Derby.

FELLOW IN OZONE RESEARCH in the Division of Meteorological Physics, Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia, to take charge of the programme of research into the measurement of ozone distribution and the meteorological applications thereof—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 420/161.

FEMALE SCIENCE ASSISTANT (preferably recent honours B.Sc. graduate), for a three-year post in bacteriology at the Dysentery Reference Laboratory—The Director, Central Public Health Laboratory, Colindale Avenue, London, N.W.9.

GRADE B ASSISTANT IN THE DEPARTMENT OF SCIENCE TO TEACH CHEMISTRY mainly to G.C.E. "A" level and in O.N.C. courses—The Principal, Reading Technical College, King's Road, Reading.

GRADUATE (metallurgy, chemistry or physics, and preferably some language knowledge) IN THE INFORMATION DEPARTMENT, for work including abstracting, production of the Association's monthly Bulletin, answering inquiries and related matters—The Secretary, British Non-Ferrous Metals Research Association, Euston Street, London, N.W.1.

GRADUATE RESEARCH ASSISTANT IN THE DEPARTMENT OF ZOOLOGY AND COMPARATIVE PHYSIOLOGY, to work on the physiology of tissue changes during metamorphosis in insects—The Registrar, The University, Edgbaston, Birmingham 15.

LABORATORY TECHNICIAN (with experience of working with scientific or technical apparatus), to assist in the Physics Laboratories—The Headmaster, Chatham House Grammar School, Ramsgate, Kent.

LECTURER IN ZOOLOGY; a LECTURER IN ORGANIC CHEMISTRY; and a LECTURER IN BACTERIOLOGY at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1, or The Registrar, University of Queensland, St. Lucia, Brisbane, Australia.

LECTURER (with practical experience and a good honours degree) IN FLUID MECHANICS—Prof. C. M. White, Imperial College of Science and Technology, London, S.W.7.

LINACRE PROFESSOR OF ZOOLOGY—The Registrar, University Registry, Oxford.

OFFICER, Assistant Experimental Officer grade (with a degree in science or horticulture, or a diploma in horticulture), to assist in

experimental work—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick.

Postdoctorate Fellow in the Department of Physics, for work on electron resonance—The Registrar, University College of North Staffordshire, Keele, Staffs.

Research Assistant (Chemist) in the Department of Chemical Pathology, for work on problems associated with steroid metabolism—The House Governor, St. Mary's Hospital, Paddington, London, W.2.

Research Assistant (with a first- or second-class honours degree in chemistry with physiology in the final year of the honours degree) in the Department of Physiology and Pharmacology—Clerk to the Governors, Chelsea College of Advanced Technology, Manresa Road, London, S.W.3.

Senior Lecturer (qualified to direct research) in Physics; a Lecturer (preferably with an interest in mycology, ecology or cytogenetics) in Botany; and a Lecturer (preferably with qualifications in research) in Mathematics—The Clerk, Northern Polytechnic, Holloway, London, N.7.

Senior Research Assistant (with a Ph.D. or appropriate research experience) in the Physics Department—Prof. J. G. Wilson, Department of Physics, The University, Leeds 2.

Timber Operations Officer (under 50 years of age, with a university degree in forestry with at least three years practical experience in the control of timber exploitation on a large scale) in the Southern Cameroons, for the administration and management of all timber operations—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.61/603/02/T.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Ciba (A.R.L.), Limited. Technical Notes No. 210 (June, 1960) Strip Heating, Part 2 (Conclusion). Pp. 6. (Duxford: Ciba (A.R.L.), Limited, 1960.) [87

The Church Information Office. Both Old and New: Fourteenth Report of the Central Council for the Care of Churches. Pp. 7+14 plates. (London: Church Information Office, 1960.) 3*s.* 6*d.* [87

Ministry of Power. Reports of H.M. Inspectors of Mines and Quarries under the Mines and Quarries Act 1954, for 1959. Scottish Division. By H. Hyde. Pp. iv+47+3 plates. (London: H.M. Stationery Office, 1960.) 3*s.* 6*d.* net. [87

The Grassland Research Institute. Experiments in Progress No. 12: Annual Report for 1958–1959. Pp. 112. 7*s.* 6*d.* The Development and Work of the Grassland Research Institute. By William Davies. Pp. ii+31+4 plates. (Hurley, near Maidenhead: Grassland Research Institute, 1960.) [117

The Chemical Society. Special Publication No. 14: Handbook for Chemical Society Authors. Pp. vii+224. (London: Chemical Society, 1960.) 21*s.*; 3 dollars. [117

Annual Report of the Yorkshire Philosophical Society for the year 1959. Pp. 15. (York: Yorkshire Philosophical Society, 1960.) [117

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 145: Chemical Weed Control in Horticulture. Pp. 25. (Belfast: Ministry of Agriculture, 1960.) [117

Scotland. Western Regional Hospital Board: Regional Physics Department. Annual Report of the Regional Physicist for the year 1959. Pp. ii+52. (Glasgow: Western Regional Hospital Board, Physics Department, 1960.) [127

The Council for Nature. Nature and Conservation in East Anglia. Pp. 16. (London: The Council for Nature, 1960.) [127

Bulletin of the British Museum (Natural History). Botany. Vol. 2, No. 8: On the Geographical Relationships of the Angiosperm Flora of New Guinea. By Ronald Good. Pp. 203–226. 10*s.* Historical. Vol. 2, No. 4: Darwin's Notebooks on Transmutation of Species. Part 3: Third Notebook, July 15th 1838—October 2nd 1838. Edited with an Introduction and Notes by Sir Gavin de Beer. Pp. 119–150. 12*s.* (London: British Museum (Natural History), 1960.) [197

United Kingdom Atomic Energy Authority. Sixth Annual Report for the period 1st April, 1959—31st March, 1960. Pp. vi+72+4 plates. 5*s.* net. Atom 1960: an Illustrated Summary of the Sixth Annual Report from 1st April 1959 to 31st March 1960 of the United Kingdom Atomic Energy Authority, with some additional material to 1st July 1960. Pp. 34. 2*s.* 6*d.* net. (London: H.M. Stationery Office, 1960.) [197

Imperial College of Science and Technology. Telecommunication as a Social Science: Inaugural Lecture, 3 November 1959. By Dr. Colin Cherry. Pp. 105–124. 4*s.* 6*d.* Physiology Has No Frontiers: Inaugural Lecture, 1 December 1959. By Prof. H. K. Porter. Pp. 125–137. 5*s.* Superficial Chemistry and Solid Imperfections: Inaugural Lecture, 2 February 1960. By Prof. F. C. Tompkins. Pp. 139–148. 5*s.* (London: Imperial College of Science and Technology, 1960. Distributed by The Modern Book Co., Ltd., 19 Praed Street, London, W.2.) [197

Other Countries

Union of South Africa: Department of Commerce and Industries, Division of Fisheries Investigational Report No. 41: The Biology of Guano-Producing Sea-Birds—The Distribution, Abundance and Feeding Habits of the Cape Penguin, *Spheniscus demersus*, off the South-Western Coast of the Cape Province. By R. W. Rand. Pp. 28. (Reprint from *Commerce and Industry*). (Pretoria: Government Printer, 1960.) [117

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